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# Conception rate, milk production and endocrine responses of lactating Holstein cows to subtropical climates in Hawaii and Mexico

Rodney Harold Ingraham  
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Conception rate, milk production and  
endocrine responses of lactating Holstein cows  
to subtropical climates in Hawaii and Mexico

by

Rodney Harold Ingraham

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Department: Veterinary Physiology and Pharmacology  
Major: Physiology (Domestic Animals)

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1973

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## TABLE OF CONTENTS

|  | Page |
|--|------|
| INTRODUCTION .....                               | 1    |
| LITERATURE REVIEW .....                          | 2    |
| Climate Stress in Warm Environments .....        | 2    |
| Climate stress - a problem of heat balance ..... | 2    |
| Climatic factors affecting heat balance .....    | 4    |
| Ambient temperature .....                        | 4    |
| Relative humidity .....                          | 5    |
| Temperature-humidity index .....                 | 5    |
| THI-field reports .....                          | 6    |
| Diurnal THI flux .....                           | 7    |
| Radiation .....                                  | 8    |
| Radiation - field studies .....                  | 9    |
| Wind .....                                       | 10   |
| Wind - field reports .....                       | 11   |
| The Climate of Hawaii .....                      | 11   |
| Physiological Responses to Heat Stress .....     | 12   |
| Rectal temperature and heat stress .....         | 12   |
| Adrenal corticoids .....                         | 14   |
| Plasma adrenal corticoid levels .....            | 14   |
| Adrenal activity in heat stress .....            | 16   |
| Adrenal activity in reproduction .....           | 19   |
| The adrenal and lactation .....                  | 21   |

|   | Page |
|---|------|
| Thyroid hormones .....  | 22   |
| Determination of plasma thyroid activity .....  | 22   |
| Thyroid activity in heat stressed cattle .....  | 24   |
| Thyroid damage and reproduction .....   | 25   |
| Thyroid-pituitary-gonadal relationships .....   | 26   |
| The thyroid and milk secretion .....  | 27   |
| Heat stress and digestion in the bovine rumen .....   | 30   |
| Heat stress and lactation .....   | 32   |
| Reproduction .....  | 34   |
| Season and reproduction .....   | 34   |
| Heat stress and reproductive function in the male .....   | 38   |
| Heat stress and reproductive function in female cattle ...  | 40   |
| Estrus and estrous cycles in heat stressed cattle ...   | 41   |
| Mechanism by which heat stress depresses fertility<br>in the female .....   | 42   |
| Rectal temperature and conception rate (CR) .....   | 44   |
| Critical period for early embryo mortality .....  | 47   |
| Nutrition and reproduction .....  | 51   |
| Hormonal alterations in heat stress .....   | 53   |
| EXPERIMENT I: SEASONAL EFFECTS OF CLIMATE ON SHADED AND UNSHADED<br>COWS AS MEASURED BY RECTAL TEMPERATURE, PLASMA<br>CORTICOIDS, PLASMA THYROID HORMONES, MILK PRODUCTION<br>AND REPRODUCTIVE EFFICIENCY ..... | 56   |
| Objectives .....  | 56   |
| Materials and Methods .....   | 56   |
| General .....   | 56   |

|   | Page |
|---|------|
| Hormone assays .....  | 58   |
| Adrenal corticoids .....  | 58   |
| Thyroid hormone assay .....   | 58   |
| Rectal temperature .....  | 58   |
| Reproduction .....  | 58   |
| Weather data .....  | 59   |
| Statistical evaluation .....  | 59   |
| Results .....   | 60   |
| Climate .....   | 60   |
| Rectal temperatures .....   | 65   |
| Plasma adrenal corticoids .....   | 69   |
| Plasma thyroxine .....  | 84   |
| Milk production .....   | 84   |
| Reproduction .....  | 87   |
| Discussion .....  | 96   |
| EXPERIMENT II: AN EVALUATION OF THE RELATIONSHIP BETWEEN THE<br>AVERAGE TEMPERATURE-HUMIDITY INDEX OF DAYS DURING<br>THE ESTROUS CYCLE AND CONCEPTION RATE WITH EMPHASIS<br>ON THE TWO DAYS PRIOR TO INSEMINATION ..... | 107  |
| Objectives .....  | 107  |
| Methods .....   | 108  |
| Herd environments .....   | 108  |
| Mexico (MX) .....   | 108  |
| Hawaii (HW) .....   | 109  |
| Hawaii (HL) .....   | 110  |

|   | Page |
|---|------|
| Definitions .....   | 110  |
| THI .....   | 110  |
| CR .....  | 111  |
| B .....   | 111  |
| Evaluation of data .....  | 111  |
| Results .....   | 112  |
| Contrasts in herd environment .....                               | 112  |
| The CR versus B-2 response curve .....                            | 115  |
| Influence of B-2, B-1 and B on CR .....                           | 116  |
| The importance of B-2 with respect to CR .....                    | 116  |
| Influence of B-2 on the CR versus B-1 response curve .....        | 116  |
| Influence of B-1 on the CR versus B response<br>curve at MX ..... | 117  |
| Influence of B on the CR versus B+1 response<br>curve at HW ..... | 118  |
| CR versus B-16 to B+2 inclusive at HW .....                       | 118  |
| Interval to first service and between services for HW ....        | 119  |
| Selected post-calving blood values at HW .....                    | 120  |
| Discussion .....  | 168  |
| Heat stress .....   | 168  |
| Rectal temperatures and CR .....                                  | 169  |
| Acclimation and CR .....  | 170  |
| Heat stress and fertility .....                                   | 170  |
| Direct effect of thermal stress .....                             | 171  |



|  | Page |
|--|------|
| Follicular activity during the estrous cycle ...   | 173  |
| Short term effect of heat stress .....             | 176  |
| Determination of a critical period .....           | 177  |
| Early embryo mortality .....                       | 178  |
| Indirect effects of heat stress on fertility ..... | 179  |
| Depressed pituitary gonadotropin .....             | 179  |
| Plasma adrenal corticoids and thyroxine .....      | 180  |
| Nutrition and CR .....                             | 181  |
| Post-calving uterine infection .....               | 182  |
| CONCLUSIONS .....                                  | 184  |
| LITERATURE CITED .....                             | 187  |
| APPENDIX .....                                     | 203  |
| ACKNOWLEDGMENTS .....                              | 230  |

## INTRODUCTION

Hawaii ranks third among the states of the U.S.A. in average milk production per cow; however, production costs are high. A primary factor contributing to these costs has been a low reproductive efficiency in the cattle. Breeding failure is responsible for relatively high cull rates, and prolonged calving-to-conception intervals result in loss of productivity.

Climates that elicit a significant degree of heat stress in animals are known to depress reproductive efficiency. Although the dairy cows of Hawaii showed some evidence of heat stress in the late summer and fall months, clear evidence that climate stress might be an underlying factor in their poor reproductive performance was not available. Consequently, the following research was initiated to obtain information concerning this possibility.

This report deals with two projects. (1) The study of changes in rectal temperatures, plasma corticoids, plasma thyroid activity and milk production in a group of Holstein cows with respect to climate changes over a period of 5 months at the Waialeale Experimental Farm on Oahu, Hawaii. (2) The evaluation of breeding records on a large dairy on Oahu, Hawaii in relation to the temperature and humidity of the days surrounding breeding. A similar evaluation of a dairy in Mexico has been included for a comparison of results.

## LITERATURE REVIEW

## Climate Stress in Warm Environments

Many authors have reviewed the problems of the European breeds of dairy cattle in heat stressing climates (Brody, 1956; Johnson, 1965b; Mahadevan, 1966; Wiersma and Stott, 1969). Central to the problem is a high level of heat production coupled with a mechanism of heat dissipation that is relatively inefficient in hot, humid climates.

Climate stress - a problem of heat balance

The following energy budget equation summarizing the balance between heat produced by the animal and the net heat loss by the animal to its environment demonstrates, in a qualitative manner, some of the factors involved in climate stress (Blaxter, 1962; Johnson, 1965b).

$$H_L = H_e + H_n = H_p \pm H_s \pm H_w$$

The terms have the following significance:

$H_L$  = heat loss by all avenues per unit time.

$H_n$  = sensible heat loss per unit time. This term includes heat loss by radiation, conduction, and convection. Heat loss by these avenues is influenced by a number of environmental factors (ambient temperature, wind conditions and exposure to solar radiation) over which the animal may have little control.

$H_e$  = evaporative heat loss per unit time. This term may be varied by the animal as it attempts to keep  $H_s$  equal to 0 (Yeck and Kibler, 1956). The efficiency of this avenue of heat loss is, in part, dependent on the vapor pressure of the ambient air

(Cargill, Stewart and Johnson, 1962). Evaporative cooling is an important means of heat dissipation by cattle. With air and surface temperatures near or above the body temperatures, it becomes their primary means of heat dissipation.

$H_p$  = heat produced in metabolism per unit time. Among the factors influencing total heat production are the following:

1. Diet. Both the quantity and quality of the diet affect heat production. In general high fiber diets produce more heat (Brown, Jared and Stull, 1967; Leighton, 1965; Stott and Moody, 1960; Tsai et al., 1967).
2. Reproductive state and level of lactation. Brody et al. (1948) reported that a cow may double its heat production per unit surface area during its lactation peak and that heat production per unit of surface area near the end of gestation may be 40% above the metabolic level at the time of breeding.
3. Acclimation. In the process of acclimation to warmer climates an animal may reduce heat production. In part this results from reduced feed intake (Kibler et al., 1965).
4. Level of activity and emotional excitement.

$H_s$  = heat storage per unit time. An increase in  $H_s$  results in an increase in average body temperature at a rate that is directly proportional to  $H_s$ . Excess stored heat results in heat stress.

$H_w$  = heat gained or lost in cooling or warming food and water to body temperature (Cunningham, Martz and Merilan, 1964; Ingraham, 1968).

### Climatic factors affecting heat balance

Climatic factors that may contribute to hot climate stress are ambient temperature, relative humidity, their combined effect on heat dissipation, the range of the diurnal flux of ambient temperature and humidity, solar radiation, failure of air movement and precipitation.

Ambient temperature      Ambient temperature (td) may depress heat loss by convection and conduction if the temperature gradient between skin and air is narrow. Yeck and Kibler (1956) reported that at climate chamber temperatures under 10 C Jersey and Holstein cows lost 80% of their total heat production by these avenues, but at an average temperature of 32.2 C as little as 10% of the total heat production was lost by these routes. Consequently, increased quantities of heat had to be lost by evaporation as ambient temperature increased. The slope of the increase in heat loss by evaporation became steeper as ambient temperature increased and tended to be linear at temperatures above 15 C. A prediction formula for the fraction of the total heat production that was lost by evaporation (both from lungs and skin) using the above data is

$$\hat{Y} = .26 + 0.022 (td-15), df = 18, r = 0.8220.$$

Although a diurnal temperature flux was used by Yeck and Kibler (1956), their results were only slightly different from those obtained in a constant temperature study by a different method of evaluating evaporative heat loss (Kibler and Brody, 1952). These data gave the following prediction formula for the fraction of total heat production lost by evaporation at ambient temperatures above 15 C.

$$\hat{Y} = .34 + 0.025 (td-15), 7 \text{ cows, } 68 \text{ observations, } r = 0.9332.$$

Relative humidity Heat loss by evaporation is depressed by high relative humidity (RH). Cargill et al. (1962) reported that with RH between 20 and 80% (td 18.3 to 32.2 C), increased RH at a given chamber temperature were associated with decreased total heat and total vapor dissipation in a group of Holstein cows. Total vapor dissipation at 26.7 C ranged from 1.06 kg/hr at 30% RH to 0.79 kg/hr at 80% RH.

Temperature-humidity index The temperature-humidity index (THI) which combines ambient temperature and relative humidity into a comfort index (Appendix, Table 101a) has been recommended for use in evaluating the heat stressing effects of these factors on cattle (Cargill et al., 1962; Johnson et al., 1962). Kibler (1964) evaluated the response of lactating Holstein cows in the climate chamber to various constant THI values in terms of respirations per minute, rectal temperature and volume of expired air per minute. Some of their responses are listed in Table 1. Note that a mild response was increased to a moderate one and a moderate response increased to a severe one by only increasing relative humidity.

Cargill et al. (1962) reported that milk production was significantly depressed at THI between 76 and 77. Berry, Shanklin and Johnson (1964) related milk production decline to THI by the following formula which is based on climate chamber studies:

$$MDec = -1.075 - 1.736NL + 0.2474 (NL)(THI)$$

where MDec is the absolute decline in milk production in kg/day/cow and NL is the normal level of production in kg/day. Daily mean THI is used in the formula. Hahn and McQuigg (1970) developed a method for predicting summer milk production losses from this equation and local weather records.

Table 1. Qualitative responses of lactating cows to various temperature-humidity combinations<sup>a</sup>

| Response        | Ambient Temp. (C) | Relative Humidity (%) | THI  | Rectal Temp. (C) | Respirations Per Minute |
|-----------------|-------------------|-----------------------|------|------------------|-------------------------|
| Normal          | 18.3              | 50                    | 63.1 | 38.6             | 32                      |
| Mild Stress     | 23.9              | 90                    | 74.1 | 38.8             | 48                      |
|                 | 26.7              | 30                    | 71.1 | 38.7             | 45                      |
|                 | 26.7              | 50                    | 73.7 | 38.9             | 55                      |
| Moderate Stress | 26.7              | 80                    | 77.1 | 39.2             | 64                      |
|                 | 29.4              | 50                    | 77.3 | 39.3             | 74                      |
| Severe Stress   | 29.4              | 70                    | 79.8 | 39.7             | 82                      |
|                 | 32.4              | 50                    | 81.0 | 39.8             | 81                      |

<sup>a</sup>Data from Kibler, 1964.

The method involves construction of a cumulative probability curve for THI and comparing it to a scale of predicted milk production loss.

THI-field reports Hahn (1969) compared the predicted milk production losses from the above method to measured losses at several locations. He reported that correlation of predicted and actual losses for Holstein cows varied from excellent in northern Ohio to good in southern Louisiana and central Missouri. Maust et al. (1972) studied the correlation of milk production with maximum daily temperature, mean daily temperature, relative humidity and THI at Beltsville, Maryland. The THI and ambient temperature showed the closest relationship to the milk production data. Although weather conditions during the 52 days of the study were signifi-

cantly correlated with milk, milk fat percentage and energy intake, they commented that effects were generally less than reported from studies in heat chambers. They concluded that the effect of summer weather on the above parameters depended principally on the stage of lactation in which the stress occurred. Cows in mid and late lactation were more adversely affected. They also concluded that in situations where the diurnal variation in temperature was around 10 C, it appeared that maximum temperature was as satisfactory for estimating cow response as the other measures which required more effort. Mean values  $\pm$  SD for THI, maximum daily temperature, minimum daily temperature, mean temperature and RH for the experiment were  $75 \pm 4.2$ ,  $31 \pm 2.6$  C,  $22 \pm 3.0$  C,  $26 \pm 2.5$  C, and  $63 \pm 11.5\%$ , respectively.

In a field study of 100 Holstein cows in early lactation over a 2-month period in Mexico, Ingraham (1968) recorded milk production losses from heat stress using THI similar to those predicted by Johnson et al. (1962). Cows capable of producing 22.7 kg of milk per day declined 2.27 kg and 4.1 kg of milk per day at a THI of 78 and 80, respectively.

Diurnal THI flux      A narrow diurnal flux of THI is an important aspect of many heat stressing climates. Brody et al. (1955b) studied the effects of diurnal ambient temperature cycles on cows in the climate chamber. They concluded that the stressful effects of diurnal temperatures are not only the result of the average and maximal and minimal temperatures, but also the number of hours exposed to heating temperatures above 26.7 C and cooling temperatures below 21.1 C.

Temperatures below 21.1 C appear to be necessary for many animals to lose significant amounts of excess stored heat. The ability of the animal



to attain heat balance at some time during each 24-hour cycle seems critical for efficient animal performance. Mendel et al. (1971) found a significant negative correlation ( $P < 0.05$ ) between rate of gain in beef cattle and the number of hours in the day in excess of 21.1 C. Kelly and Hurst (1963) evaluated the effects of climate on reproductive efficiency in dairy cattle and concluded that there was a significant relationship between the percentage of hours in excess of 26.6 C during a 10-day period and the depression of conception rates. Ittner, Guilbert and Carroll (1954) concluded that the increase in night ambient temperature above a critical temperature for cattle was the primary factor causing body temperature to rise above normal. The rise caused a reduction in feed consumption and a consequent decrease in milk production in the dairy cattle of the Imperial Valley of California.

Kibler and Brody (1956) reported that heat production within a given diurnal cycle appeared to be adjusted to the mean temperature, but it was also influenced by acclimation effects. Heat production was highest during a -12.2 to 4.4 C diurnal cycle (humidities were between 40 and 60%) and was depressed at higher temperature cycles. Peak heat production values were reached twice a day after morning and afternoon feedings. Flattening of the afternoon heat production peaks occurred during 10 to 43.3 C and 15.6 to 43.3 C cycles because of reduced afternoon feed consumption and a tendency on the part of the cows to defer much of their eating to the early morning hours.

Radiation Thermal radiation may add a significant increment to the heat load of the cow. Kibler and Brody (1954) reported that a

500 kcal/m<sup>2</sup>/hr radiation level at 21.1 C chamber air temperature provided a heat stress to the cow equivalent to a 5.56 C increase in air temperature without radiation. Rectal temperatures began to rise at 21.1 C air temperature with a radiation intensity of about 115 kcal/m<sup>2</sup>/hr and attained values from 1.95 to 2.22 C above normal at about 480 kcal/m<sup>2</sup>/hr. At 26.7 C air temperature, rectal temperatures increased by as much as 2.78 C at about 480 kcal/m<sup>2</sup>/hr of radiation. Rectal temperatures and respiration rates were usually higher and heat production slightly more depressed at 21.1 C with full radiation conditions (500 kcal/m<sup>2</sup>/hr) than at 26.7 C with no radiation.

Radiation - field studies Ittner, Bond and Kelly (1958) monitored radiation intensities on cattle in the Imperial Valley of California and reported that on a typical August day cattle in the sun received a total radiant heat load (RHL) of 663 kcal/m<sup>2</sup>/hr. Under a shade the RHL was 454 kcal/m<sup>2</sup>/hr. They reported that this was equivalent to "reducing the mean radiant temperature of the animal's surround from 153° to 98°F". The value of shades for livestock seems to vary with the climate. Givens (1965) has concluded that the RHL on cattle in the southeast U.S.A. is greater under high shades (3.66 m) than under low ones (1.83 m). He suggests that in this climate, which often has numerous white cumulus clouds in the early afternoon, some of the radiation transversing the cloud is converted to diffused solar energy. This increases the RHL for the cow under the high shade since it is exposed to more sky. Also, the actual value of shades in this kind of warm, humid climate has been debated. McCormick, Givens and Southwell (1963) have reported that beef

cattle without shades gained as well as cattle with shades in Georgia.

A group of researchers have evaluated the physiological responses of dairy cattle to solar radiation in central Texas. Harris et al. (1960) indicated that exposure to direct solar radiation had considerable effect on body temperature and respiratory rates, but it did not cause an appreciable change in milk production. Increases in radiation were second in importance to air temperature in causing changes in body temperature for animals in the sun while increased vapor pressure was second in importance for shaded animals. Williams et al. (1960) indicated that solar radiation had a direct effect on body temperature when air temperatures were below 32.2 C but had little effect at higher air temperatures. Average maximum RHL for these experiments was  $837 \text{ kcal/m}^2/\text{hr}$  as reported in a companion paper (Shrode et al., 1960).

Wind Wind directly affects heat loss by convection and evaporation. Of these two, evaporative cooling, because of the high latent heat of vaporization, is by far the more important if the outer surface (skin and hair) is moist. If, however, the outer surface has little moisture to vaporize, the increased heat dissipating effect of increased wind is due largely to convective cooling. Thompson et al. (1954) reported that if wind was plotted against environmental temperature at low air velocity (0.4 mph), vaporization gradually increased with increasing temperature from -7.8 to 18.3 C, then more rapidly to 26.7 C when maximum vaporization was reached. But when vaporization at high velocity (8 to 9 mph) was similarly plotted the rapid increase in vaporization occurred nearer 26.7 C and continued up to 35 C. They concluded

that increasing air velocity shifted the vaporization curve to the right, extending the range of physiologically tolerable temperature to a higher environmental temperature.

Wind - field reports Ittner, Kelly and Bond (1957) reported that increased air movement by means of fans (3.7 mph as opposed to 0.63 mph for controls) increased gains in beef cattle (1.077 kg/day for fanned versus 0.586 kg/day for controls). The diurnal flux in air temperature during the experiment was between 25.6 and 39.4 C. Seath and Miller (1948) allowed cows to store heat in the sun for 2 hours after which they either wet them, exposed them to a fan or both. Cows left dry without fan showed an average decrease in rectal temperature after 0.5 hours of 0.156 C as contrasted to 0.345 C for fan alone, 0.461 C for sprinkling alone and a decrease of 0.790 C for cows receiving both sprinkling and fan. At the end of 1 hour the decrease in body temperature averaged only 0.39 C for the non-sprinkled and non-fan treatment and 1.023 C for sprinkling plus fan.

#### The Climate of Hawaii

The U. S. Department of Commerce (1967) indicates that the low land climate of Hawaii can be divided into three general categories according to geographical location as follows:

1. The windward (eastern) side of the island has more air movement, rain, more scattered clouds (classified cloudy 40 to 60% of the time) and tends to be more typically tropical. Ambient temperatures above 32.2 C are rare.

2. The leeward (western) side of the island by contrast has less air movement, less rain, more clear sky, is somewhat dryer, and tends to have slightly higher daily maximum temperatures. Ambient temperatures above 35 C are rare.
3. The north shore climate is most similar to the windward climate but tends to be a little dryer and warmer. Most of the island of Oahu, Hawaii lies between latitude 21° 20' N and 21° 40' north. At this latitude there is less variation in day length than in any other state of the U.S.A. There are 2.5 hours difference between the longest and shortest day.

The most prominent characteristic of the climate of Hawaii is its constancy. The difference in average ambient temperature between the hottest and coolest month of the year in most low land places does not exceed 5 C. August and September are the warmest months.

The trade winds are prevalent 80 to 95% of the time from May through September. Winds may be in excess of 12 mph about 50% of the time. During other months trade winds can be expected 50 to 80% of the time. Major storms occur from October through March.

#### Physiological Responses to Heat Stress

##### Rectal temperature and heat stress

McDowell (1962) suggested that the best index of cow comfort was body temperature. Rectal temperature is thought to give a relatively good indication of deep body temperature (Bligh, 1955; Brody, Dale and Stewart, 1955a). Rectal temperatures above a normal of 38.6 C are a

measure of heat storage or the inability of the animal to maintain heat balance (see section on climate stress). In brief, physiological phenomena which increase heat production (lactation, pregnancy, certain diets and physical activity) may result in increased rectal temperature if the climate is such that heat loss by the animal is depressed. Acclimation to a warm environment results in reduced heat production and increased efficiency of heat loss with a consequent adjustment of rectal temperatures nearer to normal (Johnson et al., 1967; Kibler et al., 1965). There is a diurnal flux in rectal temperature that is related to the daily ambient temperature flux and feeding (Ingraham, 1968; Kibler and Brody, 1956; Simmons, Darcy and Essler, 1965; Wrenn, Bitman and Sykes, 1961). Harris et al. (1960) reported that under most conditions air temperature appeared to be the major cause of rise in body temperature. The second most important cause of increased rectal temperature was vapor pressure in shaded animals and solar radiation in unshaded animals.

Wrenn, Bitman and Sykes (1958) have reported changes in rectal temperature during the estrous cycle with a peak occurring on the day of estrus. In a subsequent report (Wrenn et al., 1961) they stated that it was difficult to use a single, once daily temperature recording as an indicator of ovarian activity, since diurnal rectal temperature variations were of the same order of magnitude as those found in the shift from estrus to ovulation.

Johnson et al. (1963) concluded that each 0.56 C rise in rectal temperature resulted in a 1.8 kg drop in daily milk production. A rectal temperature above 39.7 C decreased TDN consumption by 0.45 kg to 0.91 kg per day.

Kibler and Brody (1956) evaluated rectal temperatures during diurnal ambient temperature cycles of 10 to 43.3 C and found that the rise in rectal temperature lagged behind that in ambient temperature by 1 to 2 hours in Holsteins and by 2 to 3 hours in Jerseys. The fall in rectal temperature to normal levels with decreasing ambient temperature required about 9 hours in the Holsteins but only 5 hours in the Jerseys.

Data from different sources appear to show a correlation between daily average THI and the average daily rectal temperature of lactating dairy cows in the THI range from 70 to 82 (Table 2).

Two of the references (Kibler, 1964; Paape et al., 1973) concerned experiments conducted at constant temperatures in a climate chamber. In the third study, (Ingraham, 1968) conducted with cows in an open corral, average THI were obtained from sling psychrometer readings taken at 2 to 4 hour intervals. Average rectal temperatures were based on four readings at specified times during the day. These average daily values from the field appear to be comparable to constant temperature data from the chamber.

#### Adrenal corticoids

Plasma adrenal corticoid levels      Plasma corticoid levels are affected by many non-specific stimuli which may cause an increase in ACTH by way of the hypothalamus (Willett and Erb, 1972). Responses to these stimuli may mask the biological effects of treatments under investigation. Manipulation of the animal for bleeding is such a stimulus. Therefore, it may be necessary to train subjects in order to moderate this effect (Bassett and Hicks, 1969).

Table 2. Linear regression terms for average THI versus rectal temperature of lactating cows from several sources<sup>a</sup>

| Reference                  | THI Range | Y-Intercept (C) | Slope | df             | Correlation Coefficient |
|----------------------------|-----------|-----------------|-------|----------------|-------------------------|
| Kibler (1964)              | 71-81     | 29.38           | .129  | 9 <sup>b</sup> | 0.9518                  |
| Ingraham (1968)            | 76-82     | 29.26           | .131  | 4 <sup>c</sup> | 0.9485                  |
| Paape <u>et al.</u> (1973) | 69-82     | 29.92           | .127  | 9 <sup>d</sup> | 0.9662                  |

<sup>a</sup>Values calculated from data from Table 5 in Kibler (1964), Tables 10 through 18 in Ingraham (1968) and Table 2 (uncycled chamber temperatures) in Paape et al. (1973).

<sup>b</sup>Each entry was the average rectal temperature of 12 lactating cows during the second week of exposure to a constant THI in the climate chamber.

<sup>c</sup>Daily average rectal temperatures (average of rectal temperatures taken at 0230, 0700, 1330, and 1900) of 25 lactating Holstein cows were grouped according to the average THI on the day that they were taken and the average rectal temperature of the group used. The data represent 29 consecutive days during which THI tended to increase.

<sup>d</sup>Each entry was the average of six cows at a constant THI in the climate chamber.

Various investigators have reported the following concerning bovine plasma adrenal corticoid levels:

1. There is a diurnal flux in plasma corticoids with highest levels occurring in the early morning hours at around 0400. (Macadam and Eberhart, 1972; Wagner and Oxenreider, 1972).
2. Pregnancy and lactation do not cause a change in the diurnal pattern (Macadam and Eberhart, 1972).
3. Stage of pregnancy, stage of lactation and level of milk produc-



tion do not affect the level of plasma corticoids (Macadam and Eberhart, 1972).

4. There are changes in plasma corticoid levels during pregnancy. Plasma corticoids increase in a linear manner from day 95 to day 200 of gestation. This is followed by a drop and another linear increase from day 215 to day 260 (Randel and Erb, 1971).
5. Plasma corticoid levels increase during the last 4 days prior to parturition (Adams and Wagner, 1970; Smith et al., 1973).
6. Lactating cows have higher levels of plasma corticoids than non-lactating cows (Wagner and Oxenreider, 1972).
7. There is a transient increase in plasma corticoids during and following milking (Paape, Schultze and Smith, 1971; Smith, Convey and Edgerton, 1972; Wagner and Oxenreider, 1972).
8. Overmilking results in a greater increase in plasma corticoids than normal milking (Paape et al., 1971).
9. Purified oxytocin preparations do not elicit an adrenal response similar to milking but vasopressin does (Wagner and Oxenreider, 1972).
10. Plasma corticoids are highest at estrus and reduced during mid-cycle (Sprague et al., 1971; Swanson, Hafs and Morrow, 1972).

Adrenal activity in heat stress      Since lactation and reproduction are depressed in heat stress and adrenal function is intimately involved with these processes, considerable emphasis has been given to the role of the adrenal in heat stress phenomena. Recent reports indicate that during heat stress adrenal function is altered from normal.

Christison and Johnson (1972) monitored plasma corticoids in three

non-lactating Jersey cows during acute (4 hr at 35 C, 30% RH) and chronic (7 to 10 weeks at 35 C, 50% RH) heat stress. With acute exposure both plasma cortisol and cortisol turnover rates increased in a parallel manner, plateauing after 2 hours and declining to normal in 1 to 2 days. The metabolic clearance rate for cortisol remained unchanged. With chronic exposure plasma cortisol levels and cortisol turnover rates were depressed. Again, the metabolic clearance rate for cortisol remained unchanged. Alvarez and Johnson (1973) reported similar plasma corticoid responses to varying periods of acute and chronic exposure to heat stress. In addition they found that although there was a transient increase in plasma corticoids followed by a decline during chronic exposure, plasma catecholamine activity, which had also increased, remained elevated during the entire period of exposure. The implications of high catecholamine levels during heat stress will undoubtedly begin a new wave of speculation.

Marple et al. (1972a) exposed swine to 32.2 C, 88% RH for 8 days and found depressed plasma corticoid levels with increased plasma ACTH levels. They suggested that there might be a change in the turnover rates of plasma corticoids. The implication here would be an increase in corticoid turnover rates which would be the opposite of what Christison and Johnson (1972) found. Marple et al. (1972a) suggested that both ACTH and corticoids should be determined in order to more accurately measure pituitary and adrenal response to stress.

Rhynes and Ewing (1973a) exposed Hereford bulls to 35.5 C, 50% RH for 7 weeks. Plasma cortisol decreased from 18.03 to 11.39 ng/ml ( $P < .05$ ) during the first day of heat exposure and remained relatively

constant for the duration of the experiment. Plasma concentrations of testosterone declined to 43% of that in the control bulls during the first 2 weeks of heat treatment ( $P < .01$ ) but rose to near control levels in subsequent weeks (Rhynes and Ewing, 1973b). Testosterone metabolic half-life, metabolic clearance rate, and 24 hour production rate were similar at the conclusion of both the control and heat stressing periods. They concluded that testicular endocrine function in these bulls was able to acclimate to this hot environment.

Lee, Beatty and Roussel (1971) subjected nine lactating Holstein cows to constant chamber temperatures of from 18.5 to 29.5 C with 65% RH for 5 periods of 7 days each. Plasma corticoids were negatively correlated with chamber temperature and body temperature and were positively correlated with milk yield and solids not fat. There was a linear ( $P < .01$ ) decrease in plasma cortisol concentrations during 21 days at 29.5 C, 65% RH.

A few field studies concerning plasma corticoids have been reported. Lee et al. (1968) evaluated the influence of shade versus no shade and high grain versus low grain rations during hot weather on the plasma corticoid levels of lactating dairy cows. This Louisiana study failed to show a significant difference in plasma corticoids for either sun versus shade or high grain ration versus low grain ration.

Stott, Wiersma and Woods (1972) monitored plasma corticoids in lactating dairy cattle under open shades with air conditioning and under conventional shades during the warm season (July through September) in Arizona. Plasma corticoids and plasma progesterone were depressed more

in uncooled cattle.

Adrenal activity and reproduction      It is ironic that interest in the adrenal as a mediator of depressed fertility grew out of the belief that heat stress increased adrenal corticoid secretion. It apparently started with a report by Robson and Sharaf (1952) indicating that ACTH or cortisone injections interrupted pregnancy in mice and rabbits. The effect appeared to be directly on the uterus since the phenomena occurred in ovariectomized rabbits maintained on progesterone and in hypophysectomized rabbits. Following this lead, Velardo (1957) administered ACTH to adrenalectomized rats, found no adverse affect on pregnancy and concluded that ACTH exerted its effect on pregnancy through the adrenal gland. Fernandez-Cano (1958a, 1958b), using acute heat exposure as a stressor, caused an increased embryo mortality in normal rats but not in adrenalectomized rats. He concluded, as Velardo had, that adrenal corticoids had been responsible for the reduced fertility.

More recent versions of the foregoing experiments in other species have not yielded conclusive results. Howarth and Hawk (1968) injected ewes with hydrocortisone before and following breeding. Climate consideration apparently was not part of the original experiment. However, in two experiments conducted during the summer and early autumn, hydrocortisone acetate injected ewes had reduced embryonic survival. No effect was found in two experiments conducted during the winter. They concluded that the effect was dependent on season in this species. From a similar experiment, Thwaites (1970) concluded that cortisol administered to non-heat stressed ewes after breeding did not affect embryo survival.

Howarth (1969) also sought to determine the effect of adrenalectomy on embryo survival in heat stressed rabbits. However, adrenalectomy with heat exposure did not improve fertility over intact, heat stressed rabbits in his experiments. Tilton et al. (1972) adrenalectomized ewes for a similar experiment. Adrenalectomized and control ewes were placed in the climate chamber at 32.2 C (RH not given) for the 5 days following mating after which they were slaughtered and their reproductive tracts removed. The experiment was replicated three times with a total of 153 ewes. The number of viable embryos in the intact ewes was not reduced significantly more than in the adrenalectomized ewes in each replication. However, there was a trend ( $P < .07$ ) in this direction when data from all three replications were pooled. Reproductive performance as measured by percentage mating, ovulation, fertilization and conception rate was not significantly influenced by adrenalectomy.

This more recent series of experiments conducted by Howarth, Hawk, Tilton and their co-workers which were intended to clarify adrenal and reproductive relationships have tended to confuse what had appeared to be a lucid concept.

Investigations with ACTH injections in the bovine have been more productive although they too make the picture more complex. Brunner, Donaldson and Hansel (1969) injected Hereford heifers with ACTH (100 units/day) on days 2 through 8 of the estrous cycle and found significantly reduced CL weights. However the treatment did not affect CL weights of hysterectomized heifers. These results led to the conclusion that a uterine luteolytic mechanism was necessary for the CL regression caused by ACTH.

Wagner, Strohbehn and Harris (1972) injected ACTH into heifers on days 1 to 8 of the estrous cycle and found reduced progesterone concentration on day 11 in the CL. Plasma progesterone increased on days 1 to 5 and decreased below normal on days 8 to 10. When ACTH was injected into ovariectomized heifers for 5 days plasma progesterone increased. Presumably it came from the adrenal gland. Gwazdauskas, Thatcher and Wilcox (1972b) also reported significant increases in plasma progesterone with ACTH injections in the bovine. Investigators are presently speculating on a possible role of adrenal progesterone in the reduced fertility of heat stressed cattle.

The adrenal and lactation      The adrenal gland is considered necessary for normal lactation since adrenalectomy inhibits lactation. However, this fact does not specify the role of the glucocorticoids since the mineralocorticoids are necessary for normal electrolyte balance in the body. Organ culture techniques are helping to find some specific roles for glucocorticoids. Rivera (1972) reported that insulin and prolactin were necessary for the induction of certain enzymes (glucose-6-phosphate and 6-phosphogluconate dehydrogenase) in mouse mammary gland cultures. However, this induced activity was more consistently maintained over 24 hours and up to 5 days if corticosterone was also present in the medium. Mills and Topper (1970) have attributed the formation of rough endoplasmic reticulum in mammary explants to adrenal corticoids.

Although the adrenal-pituitary mechanism is required for maintenance of lactation, injection of excessive amounts of ACTH and cortisol may depress milk production (Cowie, 1961). This effect is attributed to the influence of glucocorticoids on gluconeogenesis. Stöckl and Jöchle (1971)

evaluated the effects of flumethasone or dexamethasone injections on plasma amino acids and thyroid activity in lactating dairy cows. Both steroids mobilized significant amounts of glucogenic amino acids. Thyroid activity was also significantly increased. Brethour (1972) reported that injections of 10 to 20 mg of dexamethasone at least 33 days prior to slaughter increased marbling scores in stilbesterol-fed beef cattle. Environmental temperature did not affect the response. Insulin did not augment or duplicate the results of the corticoid. Five grams of iodinated casein per head daily for 3 days in addition to dexamethasone decreased marbling score. These papers support the theory that the glucocorticoids have a role in determining the distribution of the nutrient intake in the animal. Possibly excessive amounts direct the nutrients away from milk.

### Thyroid hormones

Determination of plasma thyroid activity      Several in vitro methods for evaluating plasma thyroid activity have been employed. Each has some limitations. Protein bound iodine (PBI) has been the method used in most of the available literature on cattle. It gives a measure of the iodine bound to plasma proteins. Its primary shortcoming is a failure to distinguish between dietary iodine and that from the thyroid hormones (Swanson and Miller, 1973). Evaluations of climate and thyroid function by this method in cattle have not given consistent results (Blincoe and Brody, 1955a). Murphy and Jachan (1965) suggested a serum thyroid hormone determination (T4) based on the specific binding properties of thyroxine binding globulin (TBG). It is not affected by dietary iodine. A third test,

the T3 resin sponge uptake test (T3 uptake) assesses availability of binding sites for thyroxine on serum proteins. In hyperthyroidism the primary thyroxine binding sites are nearly saturated and the T3 uptake value is increased over normal. In hypothyroidism the binding sites are relatively unsaturated and the T3 uptake value is decreased. The quantity of TBG, and therefore, the number of available binding sites, is affected by the quantities of estrogen and progesterone in the circulation. Because of this the T3 uptake test value may vary with the time in the estrous cycle, pregnancy or hormone treatment. The pregnant female has more TBG and consequently test results would indicate a hypothyroid condition. Refetoff, Robin and Fang (1970) reported similar values for the cow and the human based on these methods. An estimate of PBI can be obtained if the T4 value is multiplied by 0.653. Kallfelz and Lowe (1970) evaluated use of T4, T3 uptake and PBI in the horse and found no significant difference between PBI and T4. However, there was a ninefold greater mean variance between duplicate samples by the PBI method.

The product of the T3 and T4 values of a serum sample has been referred to as the free thyroxine index. It is thought to give a measure of the free thyroxine available to the tissues. The product corrects for a situation such as pregnancy in which T4 increases and T3 uptake decreases. The index gives a euthyroid value (which would be correct for the normal animal) even though the individual values for the T4 and T3 uptake tests are abnormal.

In light of the diversity and the shortcomings of the available tests for thyroid activity, it is often difficult to compare or evaluate the literature on thyroid activity.



Thyroid activity in heat stressed cattle      Blincoe and Brody

(1955a) evaluated the effects of constant temperature, air velocity and radiation level on cows in the climate chamber. Increasing temperature from -8 to 35 C decreased thyroid activity in the cows 50 to 75%. Air velocities less than 16 km/hr (10 mph) had no measurable effect on thyroid activity. The addition of 388 kcal/m<sup>2</sup>/hr of radiant energy depressed thyroid activity. The trends in thyroid activity paralleled the trends in heat production. In a similar study with diurnal cycles (Blincoe and Brody, 1955b) a temperature cycle of 21 to 38 C decreased thyroid activity by about 30% below its value during a 4 to 21 C cycle. All tests were conducted in vivo with <sup>131</sup>I.

Johnson and Kibler (1963) reported that thyroxine <sup>131</sup>I disappearance rates in cattle were depressed at 29.4 and 35 C and that the influence of high humidity at these temperatures in decreasing disappearance rates was obvious. Disappearance rates showed some decline at THI below 71 and a marked decline above THI 75. The results nearly paralleled a reduction in feed intake and were inversely related to rectal temperature. Generally, the condition that produced a change of approximately .55 C in rectal temperature evoked a significant change in thyroxine <sup>131</sup>I disappearance rates and feed intake. Johnson and Yousef (1966) evaluated thyroxine <sup>131</sup>I disappearance rates in fasted, heat stressed cows and concluded that thyroid function is dependent on environmental temperature and not feed intake. Mitra and Johnson (1972) reported that there was a 30% increase in the biological half-life of thyroid stimulating hormone (TSH) which indicated a slower utilization by the thyroid gland at 39 C, RH 50% as compared to 18 C, RH 50%.

Thyroid damage and reproduction Brody and Frankenback (1942)

thyroidectomized six calves, one of which survived and was the subject of a research bulletin. She was not found in estrus until she was placed on a thyroprotein supplement at approximately 3.5 years of age. Spielman et al. (1945) also reported that completely thyroidectomized cows failed to manifest the normal physical signs of estrus. However, they had normal ovarian function and four that were bred conceived (two on the first service and two on the second). They had been thyroidectomized for periods of from 176 to 224 days at the time of insemination.

Doubting the validity of comparing thyroidectomized animals of long standing with the transient thyroid depression that occurs during the summer season, Williams and Stott (1966) thyroidectomized six heifers and two lactating cows and bred them on their first postsurgical estrus. The interval to breeding varied from 10 to 31 days. Since all but one of the animals conceived on first service, their results did not support the theory that low thyroid secretion was the cause of low breeding efficiency during hot weather.

Miller and Swanson (1969) caused partial thyroid destruction in one member of each of 9 pairs of identical twin dairy heifers with  $^{131}\text{I}$ . Twin pairs were bred beginning 2 months after treatment. Although there was some difficulty in detecting estrus, reproduction in thyroid damaged cows was not adversely affected. One cow gave birth to a set of twins. Milk production in thyroid damaged cows was depressed with lower initial yields and reduced persistency. Garner et al. (1961) conducted a similar experiment after  $^{131}\text{I}$  thyroid damage and also found no damage to fertility.

Lucas, Brunstad and Fowler (1958) reported that feeding 0.15 % thiouracil (a goitrogen) to gilts did not affect ovulation rate, fertilization rate, embryonic mortality during the first 25 days of gestation, or the number of pigs born alive, but gestation was lengthened significantly. Thyroprotein feeding had no effect on these parameters. In a subsequent experiment 0.15% thiouracil in the diet fed to gilts 38 days pregnant resulted in increased embryonic mortality (Brunstad and Fowler, 1959).

Since thyroid activity is low in the heat stressed animal, it has been assumed by some to be the cause of at least part of the problems of detecting estrus during the warm season. Hall, Branton and Stone (1959) studied the estrous cycles of dairy cattle in Louisiana and found that although PBI values in the cattle were inversely related to ambient temperature and humidity, anestrus was not a seasonal problem under good management.

Thyroid-pituitary-gonadal relationships      The preceding section clearly indicates that a normal level of thyroid activity is not necessary for the reproductive process in the bovine. After reviewing the literature on the thyroid and reproduction, van Tienhoven (1968) concluded that neither mild hypothyroidism nor mild hyperthyroidism are incompatible with normal or near normal reproduction whereas both severe hypothyroidism and severe hyperthyroidism reduce reproductive function, at least in some species. He also commented that both thyroidectomy and administration of goitrogens have given conflicting results even within the same species and emphasized the inconsistencies of reports involving these procedures in rats.

Nalbandov (1964) commented on the effects of thyroid states on reproduction in the female and suggested that it was not possible to generalize from one species to another or even draw conclusions that will hold true within the same species. Since most available knowledge concerning pituitary-thyroid-gonadal relationships concerns rodents and birds this warning may be apropos.

Sex steroids have been shown to have a dramatic effect on thyroid activity in the rat. Serum TSH levels and thyroid activity are reduced in gonadectomized rats (both sexes) but not in the guinea pig (D'Angelo, 1966). Brown-Grant (1966) has reported that the  $^{131}\text{I}$  uptake can be affected by estradiol in ovariectomized rats. Yamada et al. (1966) reported increased thyroid activity following injection of estradiol benzoate in both male and female rats. D'Angelo (1968) found sex differences in TSH secretion and adrenal function in rats with chronic administration of estradiol benzoate. In females there was relatively little change in adrenal function, but plasma TSH titers declined and were accompanied by indications of reduced thyroid secretion. The response in the male was biphasic according to the dose of estradiol. Lower doses produced adrenal hypertrophy and increases in adrenal corticoid concentrations. At higher doses indices of adrenal activity remained normal. Plasma TSH levels were not significantly altered in the male rat.

The thyroid and milk secretion Cowie (1961) reviewed the role of the thyroid in normal lactation and concluded that the thyroid gland was not absolutely essential for milk secretion, but that in its absence the intensity and duration of lactation was decreased. Miller and Swanson

(1969) produced thyroid damage with  $^{131}\text{I}$  in one of each of nine pairs of identical twin dairy heifers prior to breeding. Their results are presented in Table 3. The lower milk yields of thyroid damaged cows resulted from a combination of lower initial yield and reduced persistency. Thyroprotein was fed to these animals in the second and third lactation to determine if normal milk production could be obtained (Swanson and Miller, 1973). Animals fed thyroprotein continuously, beginning in the prepartum period, maintained milk production equal to their control twins. However, if thyroprotein was not started until 6 to 8 weeks after calving, milk yields of 70% of normal were maintained for 9 weeks after which they declined gradually to about 50%.

Aside from the ability of the thyroid hormones to increase the metabolic level of the animal there is no evidence of a specific role for them in lactation. Thyroactive materials stimulate increased milk production in both euthyroid and hypothyroid cows. Moore (1958) has reviewed the subject of feeding thyroprotein as a milk stimulant. Present knowledge does not support its routine use. Stanley and Morita (1967) fed thyroprotein to lactating cows for a 6 week period in a subtropical climate. Milk production was increased during this short period but significant weight loss by the cows also occurred. Thyroprotein cows had increased rumen propionate and decreased butyrate. They also had significantly higher rectal temperatures.

Excessive feeding of thyroactive materials may depress lactation. Sinha and Schmidt (1970) fed approximately 10 times the normal secretion rate of thyroxine to rats. Pituitary gland weight was not affected but

Table 3<sup>a</sup>. Thyroid activity, heart rate, and milk yield of <sup>131</sup>I irradiation-damaged cows expressed as percentages of their control identical twins

| Cows                            | Average dose, uCi <sup>131</sup> I/kg body weight |      |                   |
|---------------------------------|---|------|-------------------|
|                                 | 176   | 151  | 105               |
| Thyroid <sup>131</sup> I uptake | 8.2   | 6.4  | 85.9 <sup>b</sup> |
| Thyroxine secretion rate        | 31.8  | 24.0 | 70.5 <sup>c</sup> |
| Plasma PBI                      | 31.0  | 30.3 | 71.8 <sup>c</sup> |
| Heart rate                      | 81.8  | 84.1 | 87.2              |
| Milk yield                      | 47.3  | 50.0 | 83.6 <sup>d</sup> |
| 4% FCM yield                    | 48.4  | 46.7 | 80.1 <sup>d</sup> |

<sup>a</sup> Miller and Swanson (1969)

<sup>b,c</sup> Differs from other values in the same line: b, P<.10; c, P<.01. Other values do not differ (P<.25) from values in the same line not bearing a superscript.

<sup>d</sup> Excludes Pair 8 with abnormal lactations.

adrenal size was greatly increased as well as plasma corticoids. They concluded that hyperthyroidism impairs lactation by causing a hormonal imbalance.

Nicoll and Meites (1963) reported that either thyroxine or triiodothyronine added to the medium of organ cultures of the rat adenohypophysis resulted in a pronounced stimulation of prolactin secretion. A direct action of the thyroid hormone on the adenohypophyseal cells was demonstrated. Of the hormones tested, only estrogen and thyroid hormones were capable of stimulating prolactin secretion in the rat. Convey et al. (1973)

injected synthetic thyrotropin releasing hormone (TRH) into lactating cows and determined significant increases in thyroxine, prolactin and growth hormone. This may indicate that those factors which tend to increase thyroid production (short of the need for excessive heat production to maintain body temperature) foster a higher level of milk production.

The lower milk fat in hypothyroid cows may be due to the involvement of thyroid hormone in lipid metabolism (Fain and Wilhelmi, 1962). Greenbaum et al. (1967) found that thyroidectomy decreased the content of short-chain fatty acids and increased the content of long-chain fatty acids in the mammary glands of lactating rats.

#### Heat stress and digestion in the bovine rumen

Many factors, including the quantity and quality of the diet and physical form of the diet, may alter the rate of volatile fatty acid (VFA) production in the rumen (Meyer, Kromann and Garrett, 1965). High fiber diets tend to produce a higher proportion of acetic acid (Elliot and Loosli, 1959), result in a higher heat production (Armstrong and Blaxter, 1957) and yield less milk with a higher fat percentage (McCullough, 1966). In contrast, low fiber diets of equal caloric content tend to produce a higher proportion of propionic acid, result in a lower heat production and yield more milk with a lower percentage of fat. Brokken (1971) had proposed a system for formulating beef rations with varying levels of heat increment for animals in different climates.

Elliot and Loosli (1959) reported the following correlations between

ration fiber content, rumen acetic acid, rumen propionic acid and efficiency of milk production. Each comparison is followed by its linear correlation coefficient.

|  |      |
|--|------|
| Crude fiber content of ration versus molar percentage acetic acid    | +.89 |
| Crude fiber content in ration versus molar percentage propionic acid | -.84 |
| Net production efficiency versus molar percentage propionic acid     | +.83 |
| Net production efficiency versus molar percentage acetic acid        | -.76 |
| Net production efficiency versus acetic/propionic ratio              | -.80 |

Annison and Armstrong (1970) have recently reviewed the effect of diet on the manner in which energy is used by the body. Ensor, Shaw and Tellechea (1959) proposed that a low acetic to propionic (A/P) ratio resulted in fat being stored in the body rather than going into milk.

Davis and Merilan (1960) evaluated digestion in heat stressed (32 C, 50% RH) cattle and reported a lower feed intake with a slight increase in percentage digestion. Others have reported an altered pattern of FA production in the rumen which has stimulated investigation concerning the possibility of depression of milk in the heat stressed cow because of a change in relative proportions of acetate and propionate. Kelly, Martz and Johnson (1967), Moody et al. (1967) and Weldy et al. (1964) reported a depression of rumen VFA with the principal reduction being in acetic acid. Beyond this agreement results vary, possibly because of diet and differences in the experimental method used.



Kelly et al. (1967) monitored VFA in the rumen of cattle fed at a constant level through a fistula. With ambient temperature at 18.2 C, 50% RH, acetic acid and propionic acid were 94.7 and 33.3 mEq/liter, respectively. At 37.7 C, 50% RH they were 47.2 and 10.6 mEq/liter, respectively. This resulted in an increased A/P ratio.

Moody et al. (1967) fed cows ad libitum on a high density ration supplemented with fat and also obtained an increased A/P ratio at 32.2 C, 70% RH. They also obtained a relatively greater decline in propionic than acetic acid. The A/P ratios were 59.6 / 20.8 and 53.1 / 16.1 mEq/liter for cooled and hot treatments, respectively.

Brown, Jareed and Stull (1967) monitored rumen acid in a field experiment during the summer in Arizona. During this hot period (but not in the winter) a high barley diet resulted in increased propionate and reduced acetate in the rumen, increased weight gains and depressed milk fat percentage.

#### Heat stress and lactation

As environmental temperature increases dairy cattle consume less feed, produce less milk and milk fat and reduce heat producing functions generally (Johnson, 1965a). Johnson et al. (1967) monitored lactating cows during 9 weeks of acclimation to a constant 29 C, 50% RH in the climate chamber. The cows were not able to attain full acclimation in terms of normal feed intake, normal rectal temperature or milk yield during this period, but there was a stabilization of these parameters at 4 to 5 weeks. During the first 4 weeks the cows used body tissues to help sustain milk

production in the face of decreased feed intake, consequently less feed was consumed per unit of milk produced. Maust, McDowell and Hooven (1972) conducted a field experiment in Maryland during the warm season (June to August) in which they observed that the production of cows in early lactation was less affected by the heat because cows used body reserves rapidly to offset the impact of thermal stress. The production of cows in mid-lactation (100 to 180 days) was most adversely affected by heat stress.

It has been generally accepted that the decreased milk yields found in heat stress result from decreased feed intake. Wayman et al. (1962) and Johnson et al. (1966) reported that although cows were force fed through a rumen fistula to maintain a constant feed intake their milk production was depressed at high temperatures (31 C, 50% RH). The average decline in milk yield in a group fed ad libitum under the same temperature conditions was 3.5 kg/day compared to 2.12 kg/day for the fistulated control fed cows. Part of the extra energy available to this latter group was stored in body weight gains indicating a diversion of available energy away from milk production. The result was a decreased efficiency of dietary energy for milk production.

McDowell et al. (1969) reported that cows fed ad libitum in the climate chamber at 32.2 C, 60% RH had a decline in milk energy from lactation that was twice the reduction in digestible energy intake. Since digestibility of the ration had not been adversely affected by high temperature there was an unexplained energy loss. They suggest that the lower energy for production in heat stressed cattle results from a higher maintenance requirement because of energy used for blood transport for

cooling, increased action of the sweat glands and the increased metabolic rate owing to the increase of body temperature.

The possible effects of thyroid activity and adrenal activity on lactation in heat stressed cattle have been discussed under the individual hormone.

## Reproduction

### Season and reproduction

A seasonal flux in the fertility of cattle is well established. Its pattern varies with geographical location. Recent reports indicate that it may be different in the Bos indicus breeds than in the Bos taurus breeds of cattle (Jöchle, 1972). The flux has been attributed to three factors: (1) the seasonal changes in photoperiod, (2) the seasonal temperature changes and (3) nutritional factors associated with season. A satisfactory method of determining the contribution, if any, of each of these components to the annual fertility flux of cattle has not been developed. As would be expected, animal scientists weight them differently or completely discount one or more of them depending in part on their geographical location.

Photoperiod is known to influence sexual activity in many species (Thibault et al., 1966). However, a definite photoperiod effect on bovine reproductive activity has not been established. Mercier and Salisbury (1947) evaluated the annual fertility flux of herds in Canada, Nebraska, Indiana, Maryland, New York, Louisiana, and South China. They noted that there was a tendency for the number of services per conception

to decrease from February through spring until the days began to shorten and the hot weather intervened. In Canada no summer decrease in fertility was observed. The fertility seemed to vary directly with the length of daylight. They calculated linear correlation coefficients for conception rate versus air temperature and daylength for the month of service and the 4 months prior to breeding for the Canadian herds. These are listed in Table 4. To quote their evaluation, "Partial correlations for the second month prior to mating, using the individual observations from each herd, indicated that length of daylight and not temperature change was the most important factor associated with the conception rate under Canadian conditions. The partial correlation coefficients were 0.352 and 0.017, respectively, the first being significant at the 5% level of probability. The results suggest that longer daylight has a beneficial effect on the conception rate of cattle in Eastern Canada".

Table 4. Correlations between the conception rate and the monthly averages of climate factors for the month corresponding to and the months prior to mating<sup>a</sup>

| Correlations between<br>fertility level and: | Months for Climatic Factors |                    |                    |                    |        |
|--|-----------------------------|--------------------|--------------------|--------------------|--------|
|  | Corresponding               | Prior to mating    |                    |                    |        |
|  |                             | First              | Second             | Third              | Fourth |
| Temperature                                  | 0.588 <sup>b</sup>          | 0.732 <sup>c</sup> | 0.692 <sup>c</sup> | 0.441              | 0.220  |
| Day-length                                   | 0.275                       | 0.604 <sup>b</sup> | 0.731 <sup>c</sup> | 0.668 <sup>c</sup> | 0.460  |
| Daily sunshine hours                         | 0.346                       | 0.573 <sup>b</sup> | 0.697 <sup>c</sup> | 0.603 <sup>c</sup> | 0.388  |

<sup>a</sup> Table 2 in Mercier and Salisbury (1947).

<sup>b</sup> (P<.05).

<sup>c</sup> (P<.01).

This has perhaps been the paper most widely cited in text books supporting the theory of photoperiod effect in cattle. The validity of the conclusions has been questioned in a recent review (Clegg and Ganong, 1969). They suggested that other factors, particularly the changes in temperature associated with the season, have not been ruled out.

Thibault et al. (1966) have argued for a photoperiod effect on bovine fertility based on data from their group in France. They gave another dimension in addition to conception rate, i.e., sexual activity in terms of the interval between calving and first estrus in cattle. The interval was shortest in May and June and longest in March. Maximum fertility occurred between October and February and minimum fertility occurred in May, June and July. They concluded that neither nutrition nor temperature played a part in the seasonal variation in fertility (for their data) and suggested the importance of photoperiodism.

Whatever the cause, there does appear to be a seasonal variation in hormonal activity in cattle. Lamond (1965) observed a seasonal change in the response of beef heifers to progesterone in Australia. During the late winter and spring months the ovarian cycles were not suppressed by doses of progesterone that were satisfactory at other times of the year. Rajakoski (1960) counted follicles in bovine ovaries in a study of follicular size during the estrous cycle in Sweden. He observed the fewest number of follicles per pair of ovaries in the autumn. A significantly increased number of follicles was encountered in the winter and spring.

Jöchle (1972) evaluated seasonal fluctuations of reproductive function in Zebu cattle on the Gulf coast of Mexico. There were significantly ( $P < .001$ ) more (1200 vs. 652) conceptions during the hot (27 C, 81% RH) rainy season (June through October) than during the cooler (19 C, 80% RH), dry, but also humid season (January to May). Conception rates were 35.2% for the dry season and 64.8% for the hot, rainy season. All animals were on pangola grass pasture and were supplemented to minimize seasonal nutritional effects.

Plasse, Warnick and Koger (1970) evaluated reproductive activity in Brahman heifers on the gulf coast of Florida. There was no significant difference in occurrence of estrous periods between seasons. The incidence of undetected ovulations was significantly higher during the winter season. They reported that a negative influence of low winter temperature (5 C) on manifestation of estrus resulted in long cycles and a high frequency of undetected ovulations in this season. Nutrition was controlled in this experiment.

Steinbach and Balogun (1971) evaluated the conception rate of a herd of native cattle in western Nigeria (latitude 8°N) that maintained a mean annual conception rate of 72% over a period of 10 years. The highest number of conceptions occurred in April (7.3%) and the lowest in October (4.4%). The effect of month on conception rate was significant ( $P < .001$ ). Monthly changes in length of photoperiod, mean temperature and precipitation were evaluated in relation to a 3 month running average of conception rate. Mean monthly change in photoperiod showed the highest linear correlation with conception rate. The partial correlation coefficient of mean

change in photoperiod versus conception rate with mean temperature held constant was also significant ( $P < .01$ ). Mean temperature versus conception rate with mean photoperiod change held constant was non-significant. In this report the greatest depression in fertility occurred during the cooler period of the year (October), which was also the rainy season. These animals were not supplemented but were herded and placed in paddocks of improved grassland during the night.

Jöchle (1972) summarized the reports of 14 investigators concerning the seasons of maximum and minimum fertility for Bos indicus cattle. The only consistent finding was a seasonal flux in fertility. Nutrition was cited as part of the cause in about half of the papers. Decreasing daylength was associated with maximum fertility in three of the reports and long daylength in one report.

In contrast to the inconsistencies of the preceding reports Bos taurus dairy cattle in the southern United States are consistently reported to show a depression in fertility during the warm season which is from June through October (Hillin and Rupel, 1960; Kelly and Hurst, 1963; Poston, Myers and Ulberg, 1960; Stott and Williams, 1962). Heat stress is well established as a factor in this depression of reproductive function.

#### Heat stress and reproductive function in the male

It has been demonstrated in controlled experiments that heat stress can impair fertility in the bull. Casady, Myers and Legates (1953) concluded that impaired spermatogenesis might result from 5 weeks exposure to 30 C. Rhynes and Ewing (1973b) exposed Hereford bulls to 35.5 C, 50% RH

for 7 weeks. Plasma testosterone fell to 43% of control values during the first 2 weeks of the experiment, but rose to near control levels in subsequent weeks. Spermatogenesis, evaluated by semen characteristics and histological examination at the termination of the experiment, was seriously impaired by heat treatment.

Burfening et al. (1970) exposed male mice to 32 C, 65% RH for 24 hours. Average rectal temperature at the end of the exposure was 40.6 C. Fertilization rates dropped rapidly reaching the lowest point 18 days after stress. Embryonic survival rates decreased more rapidly. They calculated that only mature spermatozoa and late spermatids were affected by the short period of heat stress.

Several studies have indicated that sperm quality may be damaged by heating only the scrotum without a concurrent increase in body temperature. Moule and Waites (1963) evaluated semen from Merino rams after they were exposed during 3 days to 2, 6-hour periods in a climate-room at 40.5 C, 8.5 mm Hg vapor pressure and 40.5 C, 31.5 mm Hg, respectively. Semen quality was decreased in all rams after this treatment. The change in quality was related to the rise of the temperature of the subcutaneous tissue of the scrotum measured during the climate-room treatment and not to changes in rectal or flank-skin temperatures. The first ejaculate containing abnormal spermatozoa was collected 13 to 21 days after treatment. The three most severely affected rams suffered a seminal degeneration for 35 to 39 days. The same heat treatment was used again after the rams had recovered. Water at 17 to 19 C was circulated around the scrotum of some of these rams during heat exposure. Only rams exposed to heat without having the scrotum cooled produced semen of inferior



quality. Setchell and Waites (1972) have reported that local heating of the testes in rats was followed by a fall in sperm concentration in the rete testis fluid beginning 6 to 10 days later and lasting until 39 days after the heat treatment. Mazzari et al. (1968) applied a local heat treatment to the boar scrotum which raised the temperature of the testis to 39.5 or 40.5 C for 3 hours. The effect of this treatment was seen 15 to 58 days post-treatment as a severe decrease in sperm concentration and motility.

Despite the realization that heat stress can reduce male fertility it has been difficult to estimate its net effect in the field. Few bulls must endure the degree of constant heat stress used in the chamber studies. Stott (1961) evaluated the fertility of bulls in Arizona during the warm season. Their semen was used on local cows and on cows in cooler areas. Since only the local cows showed depressed fertility he concluded that the female was responsible for the depressed fertility that occurred during the warm season. From a similar evaluation in South Carolina, Kelly and Hurst (1963) concluded that males and females were contributing about equally to the low summer conception rates.

#### Heat stress and reproductive function in female cattle

In a recent review Vincent (1972) summarized the effects of heat stress on the fertility of female cattle as delaying puberty, causing anestrus, depressing estrual activity, lowering conception rates, inducing abortions and increasing perinatal mortality. Of these, anestrus (whether real or due to undetected estrus) and depression of conception rates are of the greatest economic importance to the dairyman for, in combination,

they result in abnormally long calving intervals for cows that calve near the beginning of the warm season (Poston et al., 1960).

Estrus and estrous cycles in heat stressed cattle Hall, Branton and Stone (1959) observed cows and heifers in Louisiana for 17 months to determine length of estrus, time of ovulation and the length of the estrous cycle. The average period of estrus was 11.9 hours with heifers having significantly longer estrous periods than parous cows. Since half of the estrous periods were shorter than 11.9 hours it was recommended that cows be checked for estrus at least three times a day. Anestrus did not have a seasonal distribution. However, thyroid activity as shown by protein bound iodine (PBI) closely paralleled climatic data. They concluded from this that changes in thyroid function were not involved in anestrus. Cows ovulated, on the average, 12.4 hours after the end of estrus. Cows bred 7 to 12 hours after the onset of estrus had the highest conception rates.

Gangwar, Branton and Evans (1965) found that heifers under hot climate conditions in the chamber (cycled from THI 72 to 87) had significantly shorter estrous periods than cooled animals (11 hr vs. 21 hr). For natural spring climate versus natural summer climate the periods of estrus were 20 and 14 hours, respectively. The incidence of clinical anestrus was 33% in hot cycled cows in the chamber. Intensity of estrus was also depressed under chamber heat stress. The length of the estrous cycle was increased from a normal of 20 up to 25 days under chamber heat stress.

Bond and McDowell (1972) evaluated sexual activity in beef heifers in a heat stressing chamber environment (32 C, 60% RH). Winter adapted

heifers became anestrus when placed in the chamber but, on the average, reestablished estrous cycles by the sixteenth week of heat exposure. They were then bred, conceived and delivered normal calves. Rectal palpation of the anestrous heifers indicated that the ovaries were inactive and similar to those found in heifers on low energy diets. They suggested that there might have been a reduction in the synthesis or secretion of gonadotropic hormones.

Summer adapted heifers did not become anestrus when placed in the chamber at 32 C. However, they did become anestrus at 38 C, 60% RH. They concluded from their experiments with non-lactating beef heifers that:

1. Anestrus becomes an important reproductive problem under severe heat stress.
2. Cattle are able to adapt to high temperatures if high energy, low-fiber diets and adequate water are available.
3. As cattle adapt to high temperatures, physiological processes become near normal.
4. The prior climatic conditioning of the cattle is important in their response to heat stress.
5. A sudden onset of severe heat stress causes immediate changes in the physiological processes of cattle.

Mechanism by which heat stress depresses fertility in the female

Most research on the mechanism by which heat stress depresses conception rate (CR) has been based on the assumption that the major problem is one of embryo survival in the days immediately following fertilization.

Theories concerning the reason for increased embryo mortality can be

divided into two groups. Either increased body temperature or heat stress damages the gametes directly or increased body heat alters hormonal balances or enzyme systems affecting the general environment of the oviduct and uterus reducing the chance for embryo survival.

Experiments in rabbits support the theory that body temperature may damage either sperm or ova directly. Alliston, Howarth and Ulberg (1965) incubated one-cell fertilized rabbit ova in vitro for 6 hours at a temperature corresponding to elevated body temperature (40 C) and transferred them to synchronous pseudopregnant rabbits. An increase in post-implantation embryonic mortality was found that was not observed when culture temperatures corresponded to normal body temperature (38 C) or in cultures that were begun after completion of the first cleavage. They concluded that the early rabbit embryo is directly affected by the increased maternal body temperature that accompanies thermal stress in the female.

In a similar experiment Burfening and Ulberg (1968) incubated split ejaculates of rabbit semen in vitro at 38 and 40 C for 3 hours prior to insemination into the horns of rabbits mated 4 hours previously to vasectomized males. Eggs were recovered and examined for evidence of fertilization 30 hours post coitum. There was no evidence of any effect of temperature treatment on the fertilizing capacity of the semen, but embryonic survival rate was higher (75%) in the horns inseminated with semen incubated at 38 C than those incubated at 40 C (53%). Howarth, Alliston and Ulberg (1965) performed a similar experiment in which sperm were capacitated in the uterus of rabbits held in an environment of either

32 or 21 C for 6 to 8 hours prior to transfer to test females maintained at 21 C and mated to vasectomized males 10 hours prior to sperm deposition. As in the previous experiment no significant difference was observed in fertilizing capacity between the two groups. However, there was a significant reduction in preimplantation embryo survival from the sperm capacitated in the uterus of the rabbit held at the 32 C environment.

Rectal temperature and conception rate (CR)      Several investigators have presented data indicating a relationship between rectal temperature at breeding and CR. Ulberg and Burfening (1967) indicated an almost linear decline in CR for ewes from approximately 54% at a rectal temperature of 38.5 C to 24% at 40 C. On the same graph they indicated CR for a group of cattle were 60 and 45% when rectal temperatures 12 hours after breeding were 37.5 and 38.5 C, respectively. The authors suggested that any slight increase in temperature at the time of the first few cell divisions might cause delayed death of the embryo. Long, Nipper and Vincent (1969) reported that the CR of cows with rectal temperatures below 39.7 C at breeding was 55% and significantly higher than the CR (24%) of cows bred with rectal temperatures over 39.7 C. Gwazdauskas, Thatcher and Wilcox (1972a) reported that CR of lactating cows was 53% when uterine temperature was between 37.7 and 38.2 C at the time of breeding, but CR was 25.9% if uterine temperature at breeding was between 39.4 and 40.6 C. These last two reports for cattle are in reasonably good agreement. There appears to be little doubt that the heat load of the animal pre- and post-mating can influence conception rates.

Further evidence that the depression in reproductive efficiency associated with heat stress is related to body temperature increase is offered in experiments by Elliott and Ulberg (1971) using mice. They heat stressed (32 C, 53 to 67% RH for 24 hours) females during the time when the zygotes would be undergoing the first cell division. Rectal temperatures taken at the end of the heat stressing period were negatively correlated with (1) the number of 4 and 8 cell ova/number of ovulation points recovered at 51 hours after a copulatory plug was observed; (2) the percentage of CL represented by implantation sites; and (3) the percentage of implantation sites with live fetuses at 10 days after mating. There was an indication that heat stress altered the ability of some nucleoli to take up stain and others to take up  $^3\text{H}$ -uridine. In this experiment uterine environment could not be ruled out as a cause of the high embryo mortality in the stressed group.

Thwaites (1967) evaluated the effect of heat stress on ewes acclimated to autumn temperatures in Australia. Merino and Southdown ewes were continuously exposed to elevated temperatures in a hotroom for the first 20 days of pregnancy. Hotroom temperatures were adjusted to elevate the rectal temperatures of the ewes 1.38 C and respiratory rates to 150 respirations per minute. This gave average rectal temperatures in excess of 40 C. One hundred percent embryonic death resulted. Seventy-five percent was at an early stage [defined as prior to day 11 by Edey (1967)], and did not interfere with subsequent returns to estrus after a cycle of normal length. Twenty-five percent occurred later as indicated by degenerating embryos at necropsy on day 23. In a subsequent experiment

Thwaites (1970) examined the CL, thyroid gland and adrenal glands of heat stressed ewes in early pregnancy to determine if these glands might have a role in early embryo mortality. The effect of progesterone, thyroxine and cortisol therapy on embryo survival was also evaluated. Except for evidence of depressed thyroid activity most results were negative. He proposed that changes in the luminal fluids of the Fallopian tubes and uterus were the most likely mechanism of heat induced embryo mortality in the ewe.

Dunlap and Vincent (1971) compared CR of Hereford heifers exposed to either a heat stressing (32.2 C, 65% RH) or a thermoneutral (21.1 C, 65%) environment during 72 hours post-breeding. The heifers were acclimated to the late fall or winter weather of Louisiana. No heifer in the heat stressed group conceived as opposed to 43% of the control group ( $P < .01$ ). Average rectal temperatures for the heat stressed group were in excess of 40 C after chamber exposure. Post-treatment plasma progesterone levels were positively correlated ( $P < .05$ ) with rectal temperatures. As reported in a subsequent paper, the increase in progestins over pretreatment levels was also significant ( $P < .01$ ) (Mills et al., 1972). They suggested that the increased progestins may be a factor contributing to low fertility of heat stressed cattle.

All of the chamber experiments discussed in this section support the hypothesis that rectal temperatures near 40 C shortly after breeding are associated with poor fertility. However, they all share two unnatural conditions. (1) The female was acutely heat stressed following breeding. (2) Constant, non-fluctuating stress was imposed. Previous discussions

on acclimation, adrenal corticoids and thyroid activity indicate that the hormonal picture and rectal temperature in the stabilized, heat stressed animal would be quite different from that found in the acutely stressed animal (Christison and Johnson, 1972; Kibler et al., 1965).

In efforts to provide more natural conditions, Rich and Alliston (1970a, 1970b) exposed rabbits and sheep to a daily temperature flux with 4 hours at 32.2 C, 65% RH and a minimum of 21.1 C. Rabbits were exposed beginning 18 days and sheep beginning 4 days prior to breeding. While fewer does and ewes were pregnant following exposure to the fluctuating hot environment the differences from controls at constant 21.1 C were not significant. This is a dramatic contrast to the results of acute constant stress in the previous experiments.

Critical period for early embryo mortality Dutt (1964) performed a series of experiments directed toward finding the time in relation to breeding when reproductive efficiency was most damaged by heat stress. Control ewes were kept in an open one-sided barn with an outside dry lot. Breeding occurred during November. The heat stressing environment used was 32 C, 60-65% RH. Groups were placed in the chamber on day 12 of the estrous cycle preceding breeding (before breeding group), on the day of breeding (0-day group), on the day following breeding (1-day group) and on the third, fifth and eighth days following breeding. Fertility rate was determined by slaughtering half the ewes in the control, before breeding, and 0- and 1-day groups, 3 days after breeding. The percentage of ova fertilized and the percentage normal ova were significantly depressed in ewes exposed to heat stress before breeding and on the day of breeding. The results appear in Table 5.



Table 5. Ovulation and fertility rates and percentage abnormal ova in ewes exposed to 32 C ambient temperature before, at and after breeding<sup>a</sup>

| Ewe treatment group | Number of ewes | Number of corpora lutea | Number of ova recovered | Percentage of ova fertilized | Percentage abnormal ova <sup>b</sup> |
|---------------------|----------------|-------------------------|-------------------------|------------------------------|--------------------------------------|
| Control             | 40             | 56                      | 52                      | 94.2                         | 3.8                                  |
| Before breeding     | 20             | 27                      | 27                      | 40.7 <sup>c</sup>            | 55.6 <sup>c</sup>                    |
| 0-day               | 10             | 13                      | 13                      | 69.2 <sup>d</sup>            | 46.2 <sup>c</sup>                    |
| 1-day               | 10             | 13                      | 13                      | 100.0                        | 30.8 <sup>d</sup>                    |

<sup>a</sup>From Dutt (1964).

<sup>b</sup>Includes both unfertilized and fertilized ova.

<sup>c</sup>Significantly different from control ewes,  $P < .01$ .

<sup>d</sup>Significantly different from control ewes,  $P < .05$ .

Ewes not slaughtered to determine fertility were kept in the heated room until they returned to estrus or until 24 days post-breeding. Based on lambing data, embryo mortality for ewes exposed beginning before breeding, 0-day, 1-day, 3-day, 5-day, and 8-day was estimated to be 100%, 100%, 77.8%, 61.5%, 65.4% and 38.5%, respectively. The rate of embryo mortality in ewes exposed starting on day 8 was high but not significantly higher than the control ewes. These results place the most critical period for survival of the embryo prior to the end of estrus.

Woody and Ulberg (1964) used ova transfer techniques in an effort to determine the critical period. They reported no difference in the viabil-

ity of unfertilized sheep ova transferred from donors maintained at 21.1 or 32.2 C to mated recipients maintained at a constant 21.1 C. Examination of the data indicates that the 21.1 to 21.1 C transfers had a conception rate of 35% (6 out of 17 conceived). Forty-seven percent (7 out of 15) conceived from the 32.2 to 21.1 C transfer. A few authors have cited this part of the experiment as evidence that the critical period occurs after breeding. On the contrary, the technical problems associated with embryo transfer and the extremely low CR (35%) in the ewes maintained at 21.1 C indicate that the experiment should be replicated before any conclusions are drawn especially in light of the remainder of their report. Reciprocal ova transfers of fertilized ova indicated that the reproductive tract of ewes maintained at 32.2 C did not provide as favorable an environment for embryo development as did the tract of ewes maintained at 21.1 C. Control ewes (without ova transfer) maintained in constant 21.1 C had a significantly higher level of fertility than ewes maintained in a room with constant 32.2 C. Data obtained from control ewes shifted from one air temperature to another after estrus indicated that most of the decrease in fertility due to the high air temperature had occurred by the end of estrus in these ewes. This agrees with Dutt (1964) except for the prebreeding heat exposure data obtained from ova transfer.

D'Arce et al. (1970) exposed cycling gilts to 33.3 C, dew point 15.6 or 28.9 C starting with estrus or days 4, 8, 12 or 16 post-estrus. There was no significant difference in the number of CL formed at the subsequent estrus but with the longer periods of exposure there was a tendency for reduced ovulation rate. They reported that estrous cycle length was not

affected. Protein bound iodine was depressed ( $P<.01$ ). Highest rectal temperatures occurred 6 hours after beginning of exposure and decreased till day 6. Dew point temperature had a significant ( $P<.01$ ) effect on rectal temperature.

Edwards et al. (1968) conducted three trials in which gilts were exposed to cycled (17 hr 38.9 C and 7 hr 32.2 C) periods of heat stress. No attempt was made to regulate relative humidity, but it averaged approximately 35% in the heat chamber. In Trial 1, gilts were placed in the chamber following their third estrous period, were bred at the fourth estrus, removed to outside (winter and spring) temperatures after breeding, and slaughtered between day 30 and day 35 of gestation. Gilts in the hot chamber had slightly longer estrous cycles (2 days) than controls ( $P<.05$ ) and tended to have fewer CL and viable embryos but differences were not significant.

In Trial 2, gilts were exposed to heat stress beginning 5 days prior to breeding. Results were similar to those found in Trial 1 but the length of the cycle was not significantly increased.

In Trial 3, a group of gilts was confined to the heat chamber for 15 days following breeding. These gilts had fewer ( $P<.01$ ) viable embryos and lower ( $P<.01$ ) embryo survival rates than another group of gilts exposed to the heat chamber between days 15 and 30 of gestation or still another group of gilts placed in the cool chamber (23.4 C) from breeding to day 30. This exposure to the heat chamber for the first 15 days following breeding was the most detrimental to productivity. This was an acute stress. Gilts exposed during the previous cycle (Trials 1 and 2)

had undergone some acclimation prior to mating as indicated by a return of rectal temperature toward normal.

In a later experiment this same group (Omtvedt et al., 1971) exposed gilts to 37.8 C for 17 hours at specified times during gestation. Gilts exposed during the period from day 0 to day 8 or during the period from day 8 to day 16 had fewer ( $P < .01$ ) viable embryos and slightly lower conception rates. Gilts in the latter group, which were stressed during the period of implantation, appeared to be more susceptible to heat stress. Gilts exposed during the period from day 53 to day 61 did not appear to be affected; but those exposed in the period between day 102 and day 110 farrowed fewer live pigs and had more still-born pigs. They concluded that heat stress reduced productivity most in late pregnancy which is different from Dutt's (1964) conclusions for the ewe.

#### Nutrition and reproduction

The seasonal flux in reproductive activity and efficiency has often been attributed to seasonal variations in available nutrients. Aside from the seasonal availability of nutrients some individuals have designated the voluntary reduction of feed intake that accompanies heat stress as a cause for reduced fertility during the warm season. Although this latter suggestion is unsubstantiated it is of interest that the ovaries of heat stressed cattle have been reported to be similar in size and activity to those from heifers on low energy diets (Bond and McDowell, 1972). Both conditions have been attributed to reduced synthesis and/or release of gonadotropic hormones (Bond and McDowell, 1972; Dunn et al., 1969). Madan and Johnson (1971) have reported reduced plasma LH in the

heat stressed bovine. Howland (1972) has reported reduced plasma LH in rats on restricted feed intake. Restricted feeding plus supplemental glucose resulted in a mean plasma LH concentration similar to that of full-fed controls. This, at least in part, supports the thesis that the infertility of undernutrition is due to reduced caloric intake and not to other specific components such as protein.

Dunn et al. (1969) reported that pre- and post-calving energy levels influenced the occurrence of the first postpartum estrus. Oxenreider and Wagner (1971) reported that both undernutrition and lactation delayed postpartum follicular growth and ovulation. They found a significant negative correlation between plasma glucose level and postpartum interval to occurrence of a 10 mm follicle and ovulation. Neither lactation nor energy intake appeared to exert a significant effect on normal involution of the uterus.

Stott and Thomas (1971) subjected Holstein heifers to submaintenance rations and found a pattern of high plasma corticosteroid concentration (lasting for some 30 to 40 days) was followed by subnormal levels. Fecundity was high during the period of high adrenal output of corticoids but low during the time of low plasma levels. Psychological signs of estrus did not occur in heifers having low plasma corticoid levels. Raising the nutritional intake in the starved heifers resulted in a "flushing" action with plasma corticosteroid concentrations increasing an average of five-fold. The animals showed an intensive heat on the following estrus with high fertility.

Lamond (1970) cites the high-producing dairy cow in the first month or two of lactation as a special case of undernutrition. Maust et al. (1972) evaluated milk production in relation to daily temperatures during the warm season. They emphasized that the early lactation cows were affected least by the hot weather. They consumed the least feed energy but were highest in production indicating they utilized body reserves rapidly to offset the impact of thermal stress. Wiltbank (1970), Lamond (1970), and Hollon and Branton (1971) have indicated that conception rates may be depressed in cows that are losing weight in early lactation.

Wordinger, Dickey and Hill (1972) studied the influence of undernutrition on the histology and histochemistry of the bovine endometrium. Fertilization and pregnancy rates were lower in the undernourished groups. The histological changes they found supported the thesis that there were changes in the estrogen and progesterone levels in the undernourished cows.

#### Hormonal alterations in heat stress

The following alterations in hormonal activity have been reported to occur in heat stress:

Glucocorticoid levels are depressed following a transient increase

(see section on adrenal corticoids).

Thyroid activity is depressed (see section on thyroid hormones).

Growth hormone secretion rate and plasma levels are depressed in the cow (Mitra et al., 1972). Marple et al. (1972a) reported a

slight decline in plasma levels in swine.

Adrenocorticotrophic hormone levels in the plasma of swine are reported to increase in both stress susceptible and stress resistant animals (Marple et al., 1972b).

Thyroid stimulating hormone showed a 30% increase in biological half-life indicating slower utilization by the thyroid gland (Mitra and Johnson, 1972).

Luteinizing hormone in the plasma is significantly decreased (Madan and Johnson, 1971). The interval between successive LH peaks was reported to be longer which coincided with a longer estrous cycle.

Progestins in the plasma of acutely stressed cattle were reported to increase (Dunlap and Vincent, 1971). Presumably the increase originated in the adrenal. Stott et al. (1972) reported a general decline in plasma progestins in cattle in the corral during the warm season in Arizona. At times of peak luteal function plasma progestin was also depressed suggesting depressed ovarian function. Lee et al. (1971) reported no significant change in plasma progesterone in cattle stressed in the heat chamber.

Catecholamine levels in the plasma increased at a rate correlated with rectal temperature rise and then gradually declined to pre-stress levels after about 20 days of continuous exposure in the chamber (Alvarez and Johnson, 1973).

Although hormonal imbalances have been proposed as a reason for reduced reproductive efficiency in heat stress, evidence substantiating such an hypothesis is lacking. Perhaps the most convincing evidence that hormones are involved in some manner is the consistency with which increased estrous cycle length has been associated with heat stress. Cycles of increased length have been reported by Gangwar et al. (1965) and Madan and Johnson (1971) in the bovine, Edwards et al. (1968) in swine, and Dutt et al. (1959) in sheep.



EXPERIMENT I: SEASONAL EFFECTS OF CLIMATE ON SHADED AND UNSHADED COWS AS MEASURED BY RECTAL TEMPERATURE, PLASMA CORTICOIDS, PLASMA THYROID HORMONES, MILK PRODUCTION AND REPRODUCTIVE EFFICIENCY

Objectives

Average daily THI values during the warm season on the north shore of Oahu, Hawaii are between 70 and 76. Climate stress in this THI range is not well defined in the literature. Also the value of shades in warm, humid climates of this nature has not been conclusively substantiated. Consequently, this experiment was proposed to answer the following questions:

1. Is the climate significantly affecting the dairy cows with respect to rectal temperature, adrenal corticoid levels, and thyroid function?
2. If adrenal corticoid and thyroid levels are significantly related to climate, is there evidence that they in turn may be affecting milk production and reproductive efficiency?
3. Does shade modify the effect of climate to the extent that it can be detected in the factors listed above?

Materials and Methods

General

Ten Holstein cows in early lactation were selected from the dairy herd at the University of Hawaii Waialeale Experimental Farm. Animals that tolerated haltering and gave a minimum reaction to being handled were selected.

The cows were randomly divided into 2 groups of five each. One group was tethered under sheet metal shades and the other in the sun. Both groups were on asphalt surfaces that were relatively open to air movement.

Cows were fed and watered individually. The diet consisted of a concentrate mix, pineapple bran and fresh-chopped pangola grass. Animals were fed twice daily in the corrals and at each milking in the barn. Feed was hand fed at levels approaching ad libitum feeding without having feed wastage each day. Although not measured daily, the feed consumption was in excess of the recommended feeding standards.

Cows were untied and walked to a parlor barn for milking. Milk meters were used to obtain production data at each milking.

At 2 to 3 week intervals between September and February the cows were cannulated with PE 100 polyethylene tubing in the jugular vein. Twelve ml heparinized blood samples were taken immediately prior to the morning milking (0400), before direct solar radiation was present in the corrals (0800), noon (1200), immediately prior to the afternoon milking (1600), and about sundown (2000). Samples were taken for a 36-hour period.

For the first five cannulation periods samples were immediately refrigerated and the cells removed the following day. For cannulation periods 6 to 10, samples were centrifuged and the plasma placed in the freezer within an hour after removal from the cow. Some duplicate samples were obtained during periods six and eight. The cells were immediately removed from one duplicate and 24 hr later from the other. The corticoid assays of the two duplicates were later compared.

### Hormone assays

Adrenal corticoids Plasma samples were assayed for total plasma corticoid content using the protein-binding method of Murphy (1967) as modified by Whipp and Lyon (1970) (Appendix Table 104).

Thyroid hormone assay Thyroid hormone was determined with Res-O-Mat ETR Diagnostic Kits<sup>1</sup>. The procedure (Appendix Table 107) is a combination of the T4 and T3 uptake test. Pregnancy or estrogen treatment does not influence the results. Instead of giving an absolute value for thyroxine the test compares the unknown with a human standard. The effective thyroxine ratio (ETR) is a reflection of free plasma thyroxine of the unknown in relation to that of the standard.

### Rectal temperature

Immediately after all cows were bled their rectal temperatures were taken with an electronic thermometer.

### Reproduction

Animals were observed for estrus three or more times a day. Breeding was by artificial insemination using frozen semen in the morning or during the evening.

Postpartum rectal and vaginal speculum examinations were performed to determine uterine involution and cervical discharge. Cows were medicated on the basis of these examinations. Cows that were not found in estrus were palpated periodically to determine ovarian activity.

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<sup>1</sup>Mallinckrodt Chemical Works, St. Louis, Missouri, 63160.

### Weather data

A weather station on the dairy recorded wet bulb temperatures, dry bulb temperatures and wind at 0900, 1500 and 2100. Maximum and minimum temperatures were also recorded. Hygrothermograph records were available from two other weather stations on the north shore. Solar radiation data were supplied by a sugar plantation which maintained an instrument about 3 miles from the experimental farm.

### Statistical evaluation

Data from cannulation periods three through eight (Sept. 13, Sept. 26, Oct. 10, Oct. 29, Nov. 14 and Dec. 5) were used for statistical evaluations. Cannulation periods one and two did not include all animals. A high wind damaged the corral shades in mid-December making their removal necessary so sun versus shade data were not available for cannulation periods 9 and 10.

A paired "t" test was used for shaded and unshaded group comparisons. Linear regression analysis was used in evaluations of relationships between the factors studied.

Milk production was evaluated in relation to average THI (the average of the daily average THI of the milk sampling day and the 2 preceding days) on the basis of 18 samples taken at 7-day intervals. Milk yield decline with time was separated from THI effects by means of a bivariate linear regression analysis (Steel and Torrie, 1960).

One cow in the unshaded group died during the experiment from endocarditis. It was completely eliminated from the evaluation leaving the unshaded group with only four cows.

## Results

Climate

Average daily THI declined from 75.2 in September to 70.0 in December for a total decline of 5.2 THI units (Figure 1a, Table 6). This decline showed a significant ( $P<.001$ ) linear correlation with time (the number of days from September 13). THI (at 0800 and 1600) on each of the sampling days are graphed in Figure 2a. Average maximum and minimum ambient temperatures were 28.9 and 21.9 C, respectively, for September and 25.4 and 20.1 C, respectively, for the first third of December. This relatively narrow daily temperature flux, which is equal to the difference between summer and winter average daily temperatures, is typical of Hawaii's climate.

Table 6. Mean daily THI, solar radiation and wind velocities

|  | Sept.<br>13 | Sept.<br>26 | Oct.<br>10 | Oct.<br>29 | Nov.<br>14 | Dec.<br>5 |
|--|-------------|-------------|------------|------------|------------|-----------|
| Mean THI   | 75.2        | 74.2        | 74.2       | 72.6       | 71.8       | 70.0      |
| Radiation <sup>a</sup> (kcal/m <sup>2</sup> /hr) | 229         | 207         | 203        | 159        | 106        | 117       |
| Wind (Km/hr)                                     | 15-19       | 11-13       | 9-13       | 11-28      | 19-22      | 13-37     |
| Wind (Knots/hr)                                  | 8-10        | 6-7         | 5-7        | 6-15       | 10-12      | 10-20     |

<sup>a</sup>Average for week preceding sample day.

Figure 1. Seasonal changes in the factors evaluated

- a. Average THI of each bleeding day and average solar radiation for the week preceding bleeding is plotted to show the decline as the season progressed
- b. Average rectal temperature of the day of bleeding for shaded and unshaded cows is plotted to show the decline as the days became cooler
- c. Average effective thyroxine ratio (ETR) of shaded and unshaded cows plotted to show the change as the season became cooler
- d. Average daily plasma corticoid (ng/ml) in shaded and unshaded cows plotted to show change as the season progressed
- e. Average milk production from shaded and unshaded cows plotted to show the decline as the season progressed

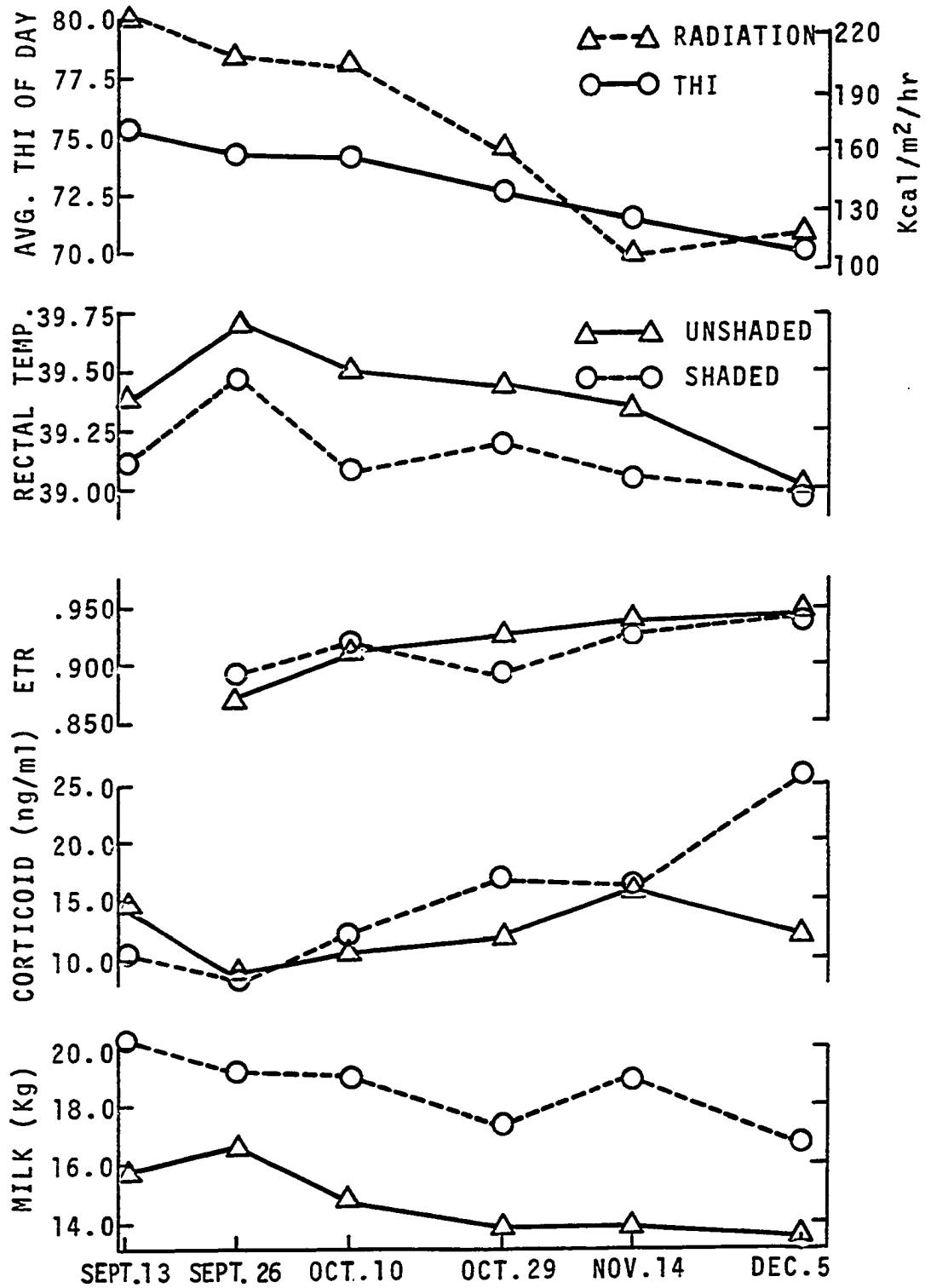
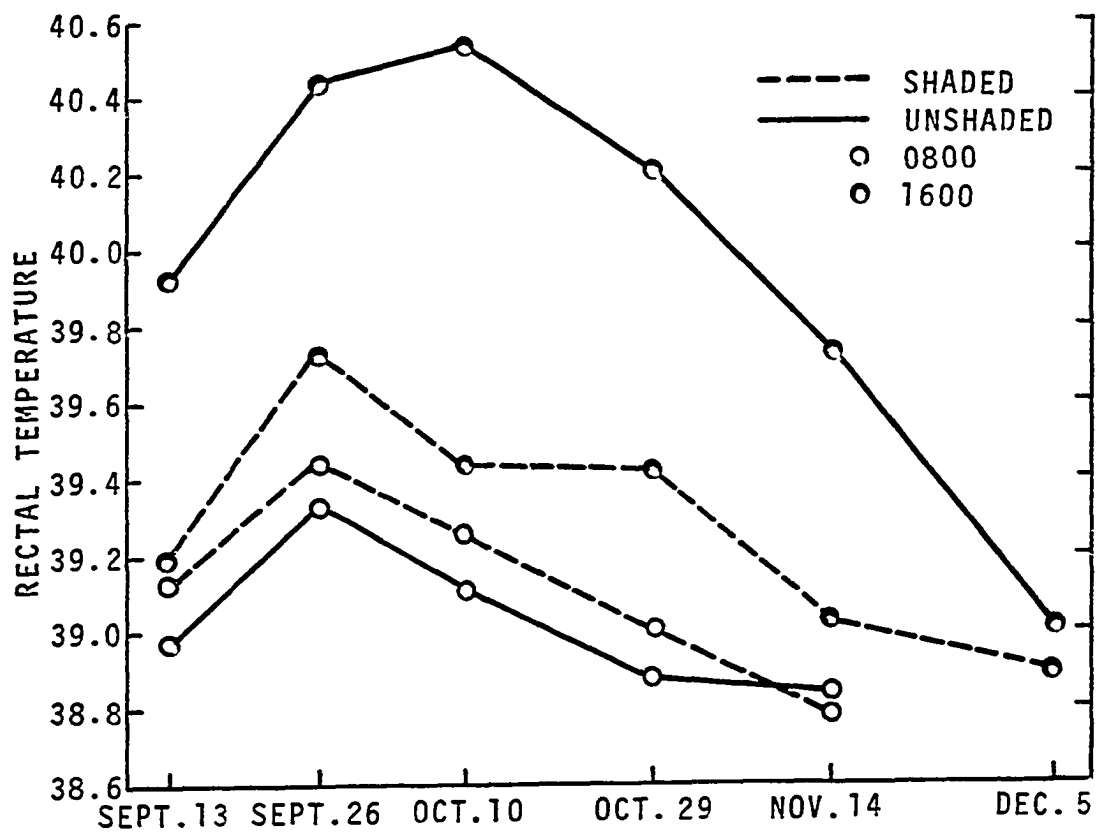
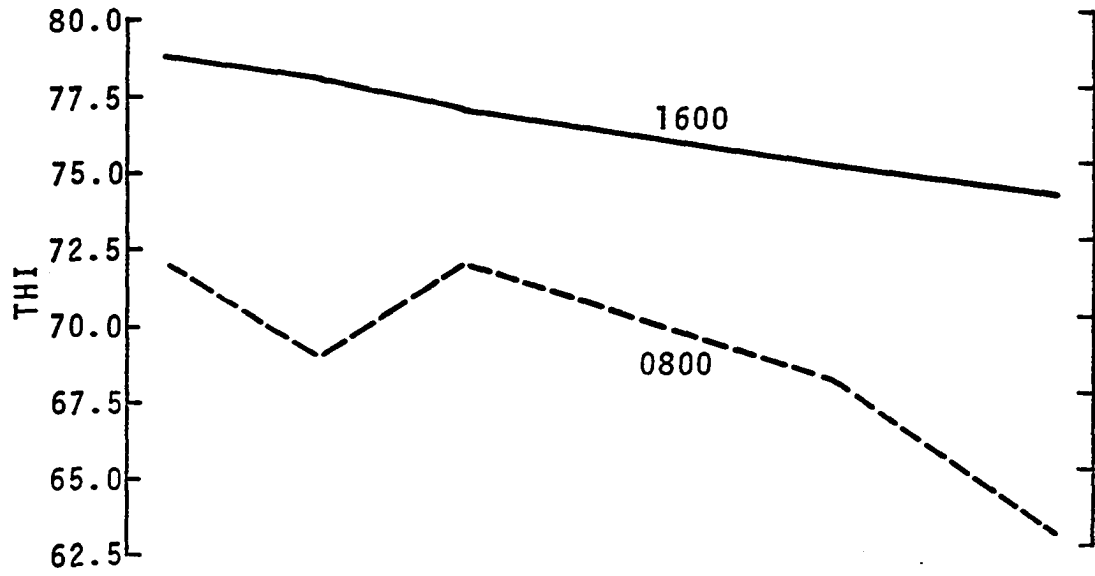


Figure 2. Seasonal changes in rectal temperature and THI at 0800 and 1600

a. THI at 0800 and 1600

b. Rectal temperatures at 0800 and 1600 in shaded and unshaded cows





The mean maximum and minimum relative humidity values ( $\pm$ SD) during the experiment were  $85 \pm 5\%$  and  $64 \pm 6\%$ , respectively. Average solar radiation declined from  $229 \text{ kcal/m}^2/\text{hr}$  on September 13 to a low of  $107 \text{ kcal/m}^2/\text{hr}$  in November (Figure 1a), the month with the most rainfall. Mean THI and solar radiation declined in a parallel manner ( $r = 0.93$ ,  $P < .01$ ) except in November which was stormy and overcast.

Winds tended to be gusty with speeds between 9 and 37 km/hr (5 to 20 mph) (Table 6). Light rain showers were common and occurred at any time of the day or night during September and October. Winter storms with significant quantities of rain began in November.

#### Rectal temperatures

Unshaded cows had significantly ( $P < .005$ ) higher daily average rectal temperatures (an average of 0400, 0800, 1200, 1600 and 2000 rectal temperatures) than shaded cows (Figure 1b). Afternoon (1600) rectal temperatures of unshaded cows were significantly ( $P < .01$ ) elevated ( $1.5 \pm .23 \text{ C}$ ) over rectal temperatures at 0800 on each of the sampling days (Figure 2b). By contrast, shaded cows showed a significant ( $P < .05$ ) increase on only one of the sampling days. Mean rectal temperatures by sample days are given in Table 7. Rectal temperatures by cow and bleeding time are given in Appendix Table 103.

Although unshaded cows gained more heat than shaded cows during the day, they lost more heat at night. This is evident in Figure 2b which indicates that unshaded cows had slightly lower rectal temperatures at 0800 than shaded cows in four of the five sampling days evaluated for this

Table 7. Rectal temperature at 0800, 1600 and daily mean for each blood sampling day in shaded and unshaded cows

|                       | Sept. 13     | Sept. 26     | Oct. 10      | Bleeding Days |              | Nov. 14            | Dec. 5 | Mean  |
|-----------------------|--------------|--------------|--------------|---------------|--------------|--------------------|--------|-------|
|                       |              |              |              | Oct. 29       |              |                    |        |       |
| Unshaded <sup>a</sup> |              |              |              |               |              |                    |        |       |
| Mean <sup>b</sup>     | 39.38 ± 0.49 | 39.69 ± 0.48 | 39.48 ± 0.58 | 39.44 ± 0.53  | 39.35 ± 0.38 | 38.99 ± 0.35       |        | 39.39 |
| 0800                  | 38.97 ± 0.42 | 39.33 ± 0.50 | 39.11 ± 0.72 | 38.87 ± 0.64  | 38.83 ± 0.37 | ----- <sup>c</sup> |        | 39.02 |
| 1600                  | 39.92 ± 0.52 | 40.44 ± 0.52 | 40.54 ± 0.57 | 40.21 ± 0.45  | 39.73 ± 0.44 | 39.05 ± 0.48       |        | 39.98 |
| Shaded <sup>d</sup>   |              |              |              |               |              |                    |        |       |
| Mean                  | 39.13 ± 0.23 | 39.47 ± 0.39 | 39.08 ± 0.19 | 39.19 ± 0.28  | 39.05 ± 0.24 | 38.96 ± 0.32       |        | 39.15 |
| 0800                  | 39.13 ± 0.36 | 39.44 ± 0.34 | 39.26 ± 0.35 | 39.00 ± 0.20  | 38.78 ± 0.39 | ----- <sup>c</sup> |        | 39.12 |
| 1600                  | 39.19 ± 0.27 | 39.73 ± 0.64 | 39.44 ± 0.34 | 39.42 ± 0.48  | 39.02 ± 0.23 | 38.89 ± 0.35       |        | 39.28 |

<sup>a</sup>n = 4.

<sup>b</sup>Mean ± SD of 0400, 0800, 1200, 1600 and 2000 rectal temperatures except for December 5 when it was the mean of 1600 and 2000 rectal temperatures.

<sup>c</sup>Thermometer inoperative.

<sup>d</sup>n = 5.

difference. Shaded cows averaged 0.81 C lower rectal temperatures ( $P < .001$ ) at 1600 than unshaded cows for the five sampling days between September 13 and November 14.

Rectal temperature at 1600 (which tended to be the high for the day) was, in part, dependent on the rectal temperature of the cow at 0800. An analysis of variance of the relationship between 0800 rectal temperature and rectal temperature at 1600 for unshaded cows is given in Table 8. The rectal temperature at 0800 and the average of the THI at 0800, 1200 and 1600 accounted for 80% of the variability of rectal temperature at 1600. The prediction formula for rectal temperature (RT) at 1600 in unshaded cows was as follows:

$$\hat{Y} = -2.55 + .160 (\text{Avg. THI}) + .786 (0800 \text{ RT})$$

A similar analysis of variance for shaded cows attributed 49% of the variance of rectal temperatures at 1600 to 0800 rectal temperatures (Table 9). Average THI contributed essentially nothing to regression in this model. The prediction formula for rectal temperature at 1600 in shaded cows was as follows:

$$\hat{Y} = 5.316 - .00038 (\text{Avg. THI}) + .872 (0800 \text{ RT})$$

There was a seasonal trend in daily average rectal temperatures which was most evident in unshaded cows (Figure 1b). The peak value in this group was 39.69 C on September 26 from which it declined to 38.99 C on December 5. In the shaded group rectal temperatures declined from 39.47 to 38.96 C during the same period.

Average daily rectal temperatures and average daily THI were significantly correlated ( $r = 0.880$ ,  $P < .01$ ) only in the unshaded group

Table 8. Analysis of variance (linear model) for influence of rectal temperature at 0800 (0800 RT) and average THI from 0800 to 1600 (Avg. THI) on the rectal temperature at 1600 in unshaded cows

| Source of Variance                                | df | SS     | MS    | F                 |
|---|----|--------|-------|-------------------|
| Regression due to both 0800 RT and Avg. THI       | 2  | 11.402 | 5.701 | 44.8 <sup>a</sup> |
| Regression due to 0800 RT, corrected for Avg. THI | 1  | 3.943  | 3.943 | 31.0 <sup>a</sup> |
| Regression due to Avg. THI, corrected for 0800 RT | 1  | 2.689  | 2.689 | 21.1 <sup>a</sup> |
| Residual  | 23 | 2.924  | 0.127 |                   |
| Total   | 25 | 14.326 |       |                   |

<sup>a</sup>(P<.0005)

Table 9. Analysis of variance (linear model) for influence of rectal temperature at 0800 (0800 RT) and average THI from 0800 to 1600 (Avg. THI) on the rectal temperature at 1600 in shaded cows

| Source of Variance                                | df | SS    | MS    | F                 |
|---|----|-------|-------|-------------------|
| Regression due to both 0800 RT and Avg. THI       | 2  | 2.288 | 1.144 | 9.54 <sup>a</sup> |
| Regression due to 0800 RT, corrected for Avg. THI | 1  | 1.816 | 1.816 | 15.5 <sup>a</sup> |
| Regression due to Avg. THI, corrected for 0800 RT | 1  | 0.000 | 0.000 | 0.0               |
| Residual  | 20 | 2.397 | .120  |                   |
| Total   | 22 | 4.686 |       |                   |

<sup>a</sup>(P<.001)

(Figure 3a). The prediction formula for average daily rectal temperature in unshaded cows was as follows:

$$\hat{Y} = 30.12 + 0.126 (\text{Avg. THI of day})$$

Relationships between rectal temperature and plasma thyroid hormones and plasma corticoids are given in Figure 4 and will be discussed in the sections on these hormones.

#### Plasma adrenal corticoids

Plasma corticoid values for shaded and unshaded cows are shown in Figure 1d. Shaded cows had a higher average plasma adrenal corticoid level (14.6 ng/ml) than unshaded cows (12.0 ng/ml). This difference was significant ( $P < .05$ ) in a paired "t" test of the mean corticoid levels for the hour of bleeding (Table 10, Figure 5), but not if the means of shaded and unshaded groups on bleeding days were compared (Table 11). Mean plasma corticoid levels for shaded and unshaded cows at each bleeding are graphed by sample day in Figure 6. Plasma samples taken just prior to milking (0400 and 1600) generally had the highest corticoid concentrations. Values at 0400 were higher ( $P < .05$ ) than those at 0800 and 2000. Mean plasma corticoid levels listed by bleeding day at 0400 and 1600 are given in Table 11. Plasma corticoid levels by cow and bleeding are given in Appendix Table 106.

Neither plasma corticoid levels prior to milking nor daily average plasma corticoids were significantly correlated with milk production in a bivariate analysis that included time (days from the beginning of the experiment).

Figure 3. Rectal temperature and hormone response to THI

- a. Average rectal temperature of sample day for unshaded group versus average THI of the sample day ( $r = 0.880$ ,  $df = 5$ ,  $P < .01$ )
- b. Average plasma corticoids of the sample day for the shaded group versus average THI of the sample day ( $r = -.920$ ,  $df = 4$ ,  $P < .01$ )
- c. Average ETR at 1600 for shaded and unshaded group versus THI 1600. Correlation coefficients, degrees of freedom and P values for shaded and unshaded cows were  $r = -.916$ ,  $df = 3$ ,  $P < .05$  and  $r = -.944$ ,  $df = 3$ ,  $P < .02$ , respectively

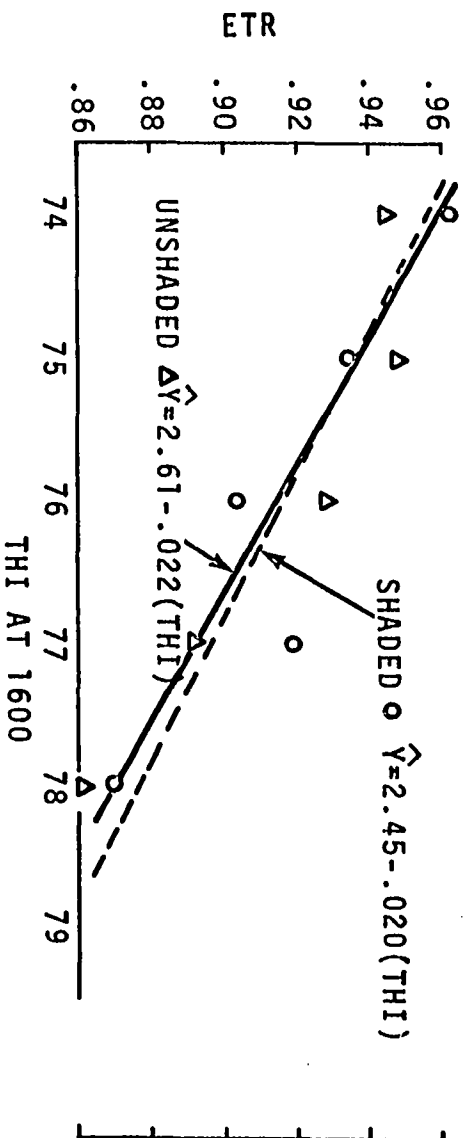
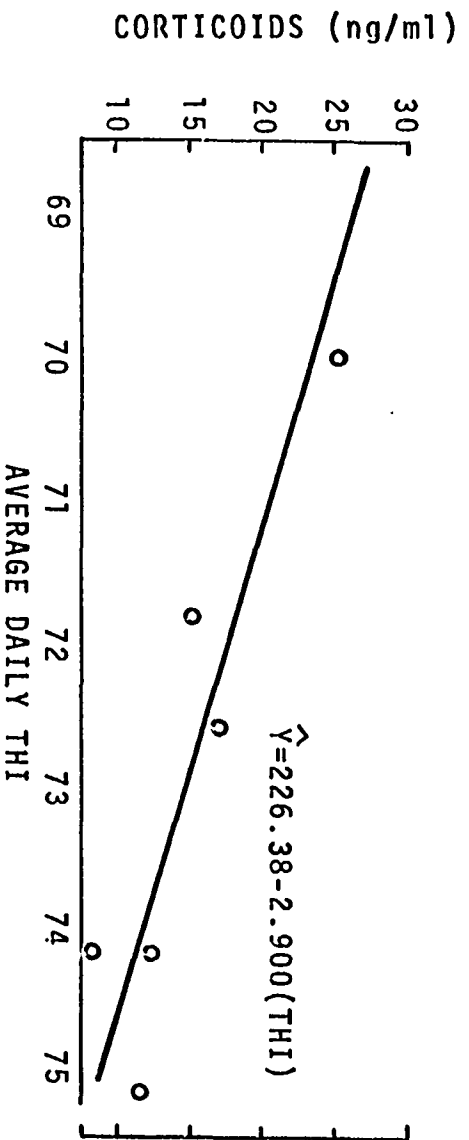
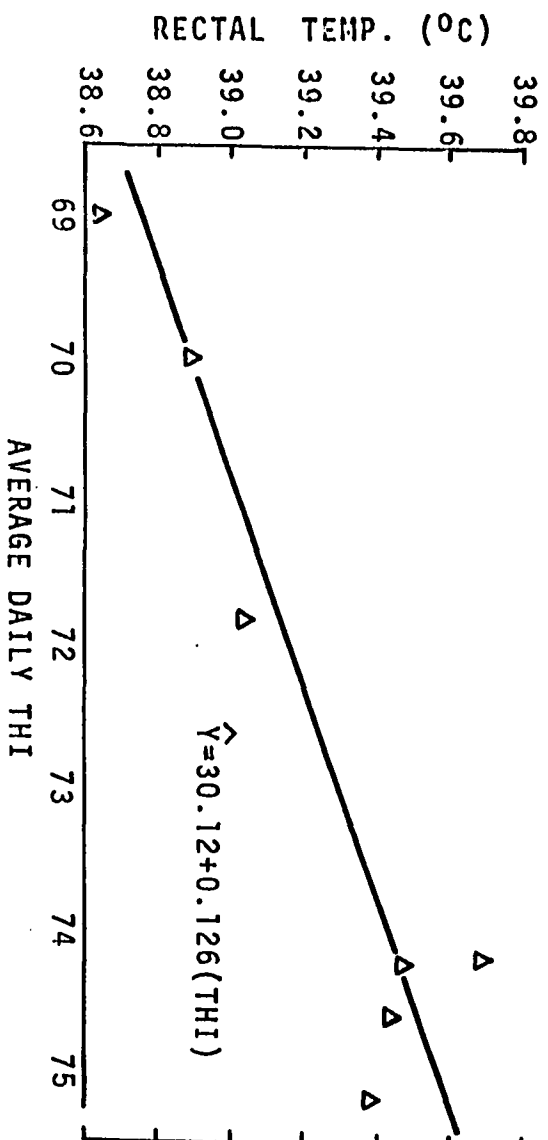




Figure 4. Hormonal response to rectal temperature

- a. Plasma corticoids at 0400 of both shaded and unshaded cows versus rectal temperatures at 0400

- b. Effective thyroxine ratio (ETR) values at 1600 of shaded cows versus rectal temperatures at 1600

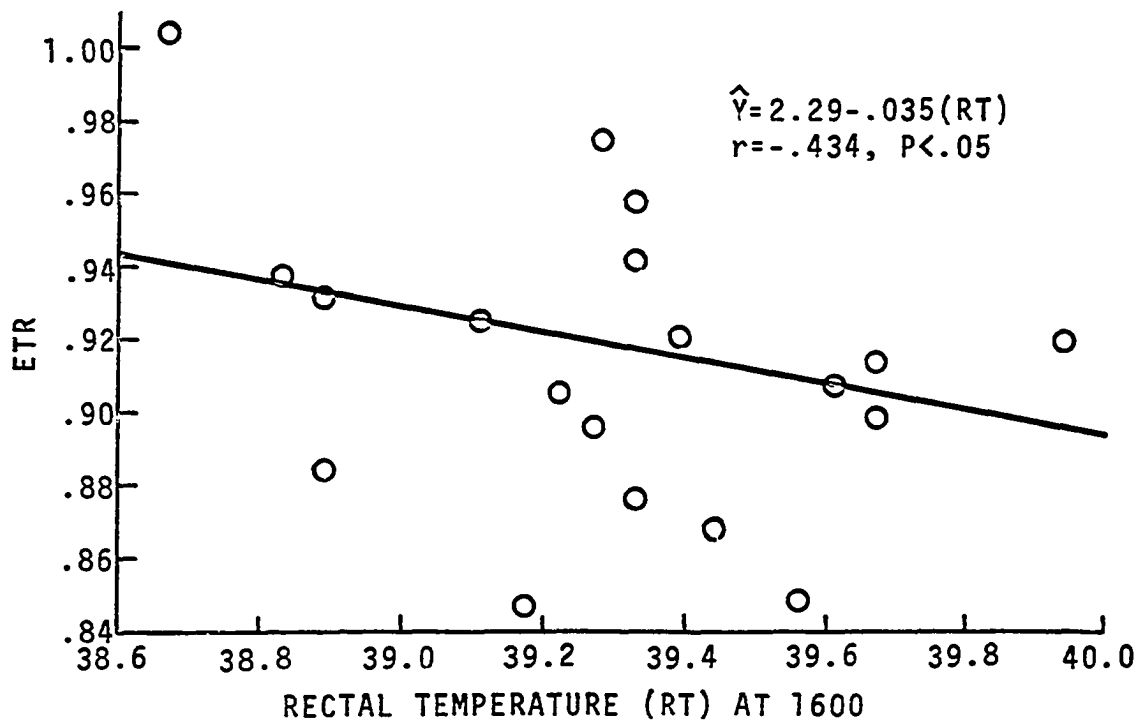
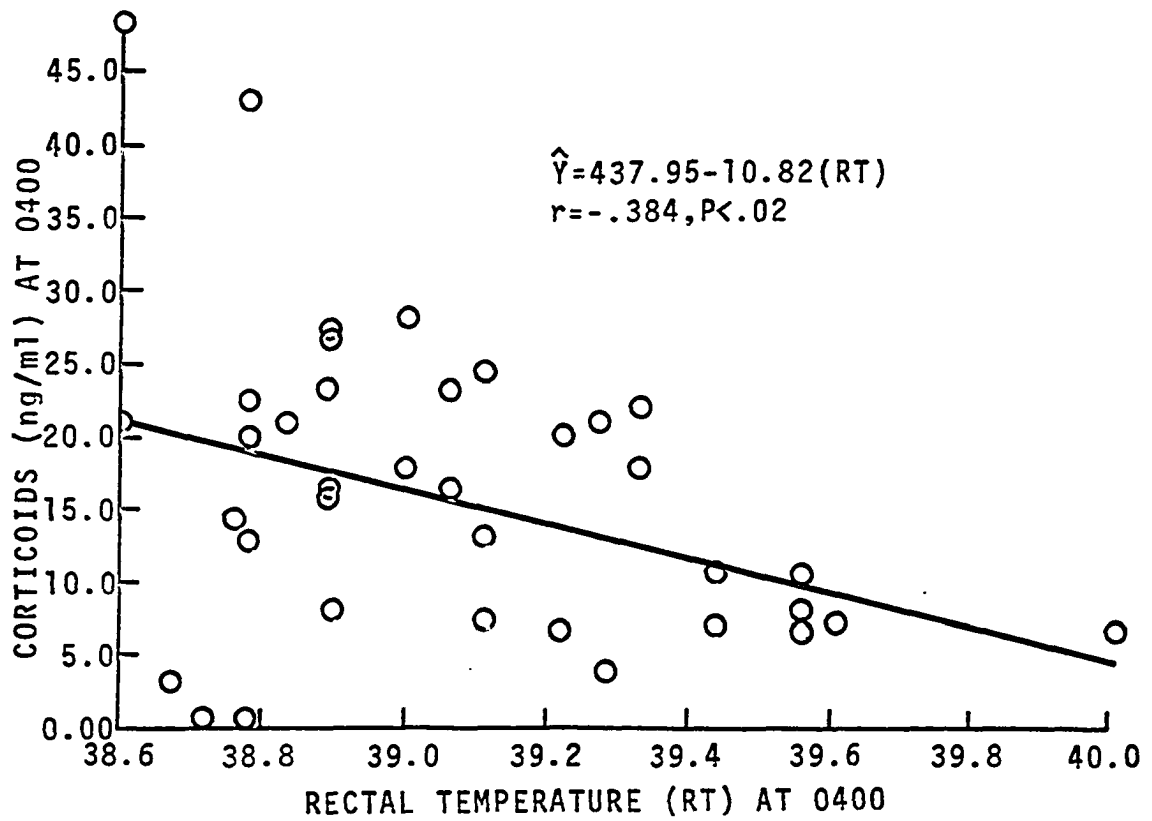


Table 10. Mean ( $\pm$ SD)<sup>a</sup> plasma adrenal corticoid levels (ng/ml) by time of day in shaded and unshaded cows

|          | Sampling Time  |                |                |                |                |      |
|----------|----------------|----------------|----------------|----------------|----------------|------|
|          | 0400           | 0800           | 1200           | 1600           | 2000           | Mean |
| Unshaded | 14.5 $\pm$ 4.2 | 12.0 $\pm$ 2.5 | 9.7 $\pm$ 4.2  | 13.0 $\pm$ 2.7 | 10.6 $\pm$ 5.7 | 12.0 |
| Shaded   | 18.4 $\pm$ 9.0 | 12.8 $\pm$ 5.1 | 12.7 $\pm$ 7.6 | 17.3 $\pm$ 8.3 | 11.7 $\pm$ 7.4 | 14.6 |

<sup>a</sup>Each value represents the mean of four cows (unshaded group) or five cows (shaded group) for 6 days. The standard deviations are between sample day means (n = 6).

Plasma corticoid levels tended to increase as the season progressed and the weather cooled. Both time (days from the beginning of the experiment) and THI showed a significant ( $r = 0.9106$ ,  $P < .02$  and  $r = -.920$ ,  $P < .01$ , respectively) linear correlation with plasma corticoid levels in shaded cows (Figure 3b) but not in unshaded cows. Although plasma corticoids increased from September 26 to November 14 in the unshaded group they declined for December 5 and had declined further to 10.1 ng/ml on December 30.

Based on pooled data from both shaded and unshaded cows, plasma corticoid levels and rectal temperatures were correlated ( $r = -.385$ ,  $P < .02$ ) (Figure 4a) at 0400. Plasma corticoid levels and plasma thyroid hormones were not significantly correlated.

Comparison of values for samples centrifuged immediately versus holding for 24 hours prior to centrifugation did not indicate a significant change in corticoid content due to standing.

Figure 5. Mean plasma corticoid levels at 0400, 0800, 1600 and 2000 for shaded and unshaded cows (pooled data for all bleeding days)

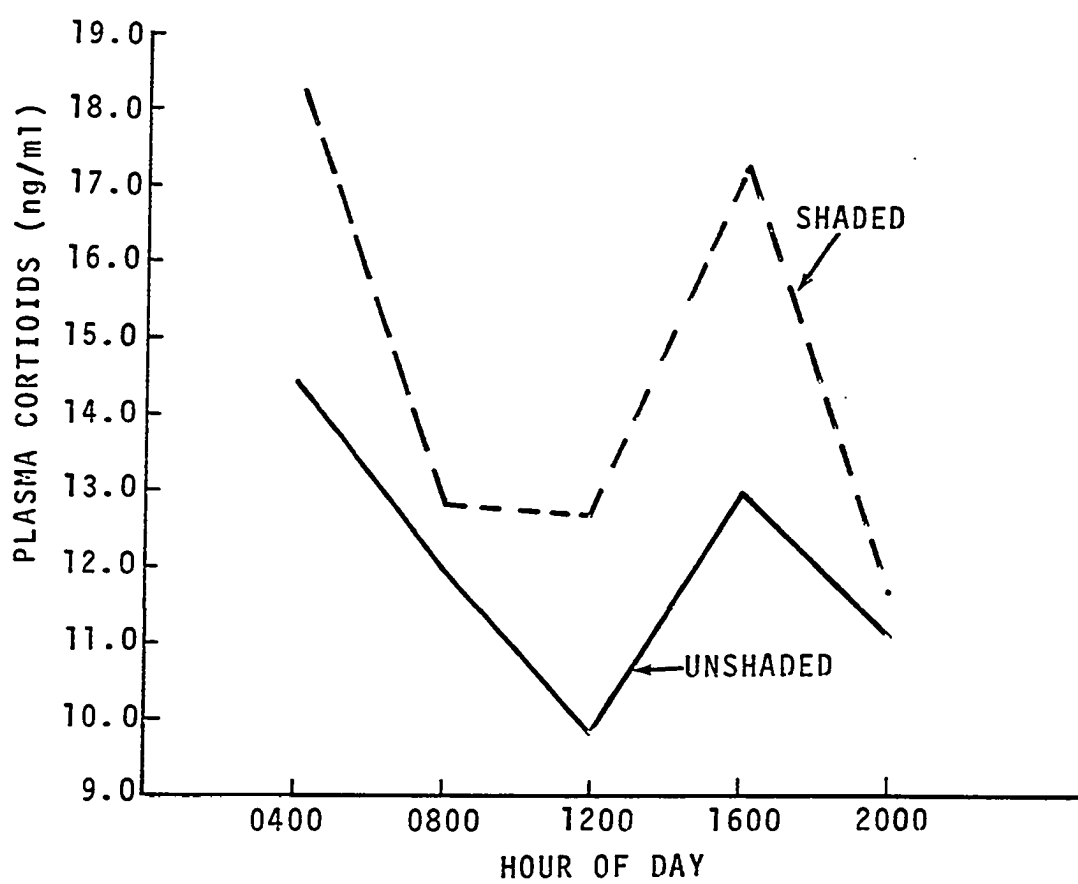


Table 11. Mean ( $\pm$ SD) plasma adrenal corticoid values (ng/ml) for the full day of bleeding and at 0400 and 1600 in shaded and unshaded cows

|                 | Sept. 13                        | Sept. 26           | Bleeding Days      |                    | Nov. 14            | Dec. 5              | Mean |
|-----------------|---------------------------------|--------------------|--------------------|--------------------|--------------------|---------------------|------|
|                 |                                 |                    | Oct. 10            | Oct. 29            |                    |                     |      |
| <b>Unshaded</b> |                                 |                    |                    |                    |                    |                     |      |
| Mean            | 14.5 $\pm$ 2.9(15) <sup>a</sup> | 8.0 $\pm$ 1.1(20)  | 10.6 $\pm$ 5.4(20) | 11.8 $\pm$ 8.5(20) | 15.6 $\pm$ 9.0(20) | 12.2 $\pm$ 3.4(20)  | 12.1 |
| 0400            | 15.8 $\pm$ 7.5(3)               | 12.2 $\pm$ 6.7(4)  | 9.9 $\pm$ 9.3(4)   | 18.3 $\pm$ 20.9(4) | 20.1 $\pm$ 17.6(4) | 10.8 $\pm$ 1.8(4)   | 14.5 |
| 1600            | 14.6 $\pm$ 8.8(3)               | 12.3 $\pm$ 3.4(4)  | 12.8 $\pm$ 7.7(4)  | 11.9 $\pm$ 4.4(4)  | 17.4 $\pm$ 12.5(4) | 9.4 $\pm$ 5.2(4)    | 13.1 |
| <b>Shaded</b>   |                                 |                    |                    |                    |                    |                     |      |
| Mean            | 10.6 $\pm$ 5.3(15)              | 8.1 $\pm$ 1.8(25)  | 12.2 $\pm$ 6.1(25) | 16.8 $\pm$ 7.6(25) | 15.4 $\pm$ 7.5(25) | 25.2 $\pm$ 12.5(25) | 14.7 |
| 0400            | 15.4 $\pm$ 7.6(3)               | 9.0 $\pm$ 8.9(5)   | 14.0 $\pm$ 8.5(5)  | 18.2 $\pm$ 9.2(5)  | 18.2 $\pm$ 10.4(5) | 35.5 $\pm$ 28.6(5)  | 18.4 |
| 1600            | 9.1 $\pm$ 5.2(3)                | 13.7 $\pm$ 10.0(5) | 15.5 $\pm$ 11.7(5) | 22.7 $\pm$ 11.6(5) | 11.5 $\pm$ 3.6(5)  | 31.6 $\pm$ 27.3(5)  | 17.3 |

<sup>a</sup>( ) Number of samples averaged.

Figure 6. Mean plasma corticoid levels for shaded and unshaded cows at each bleeding on each of the sample days

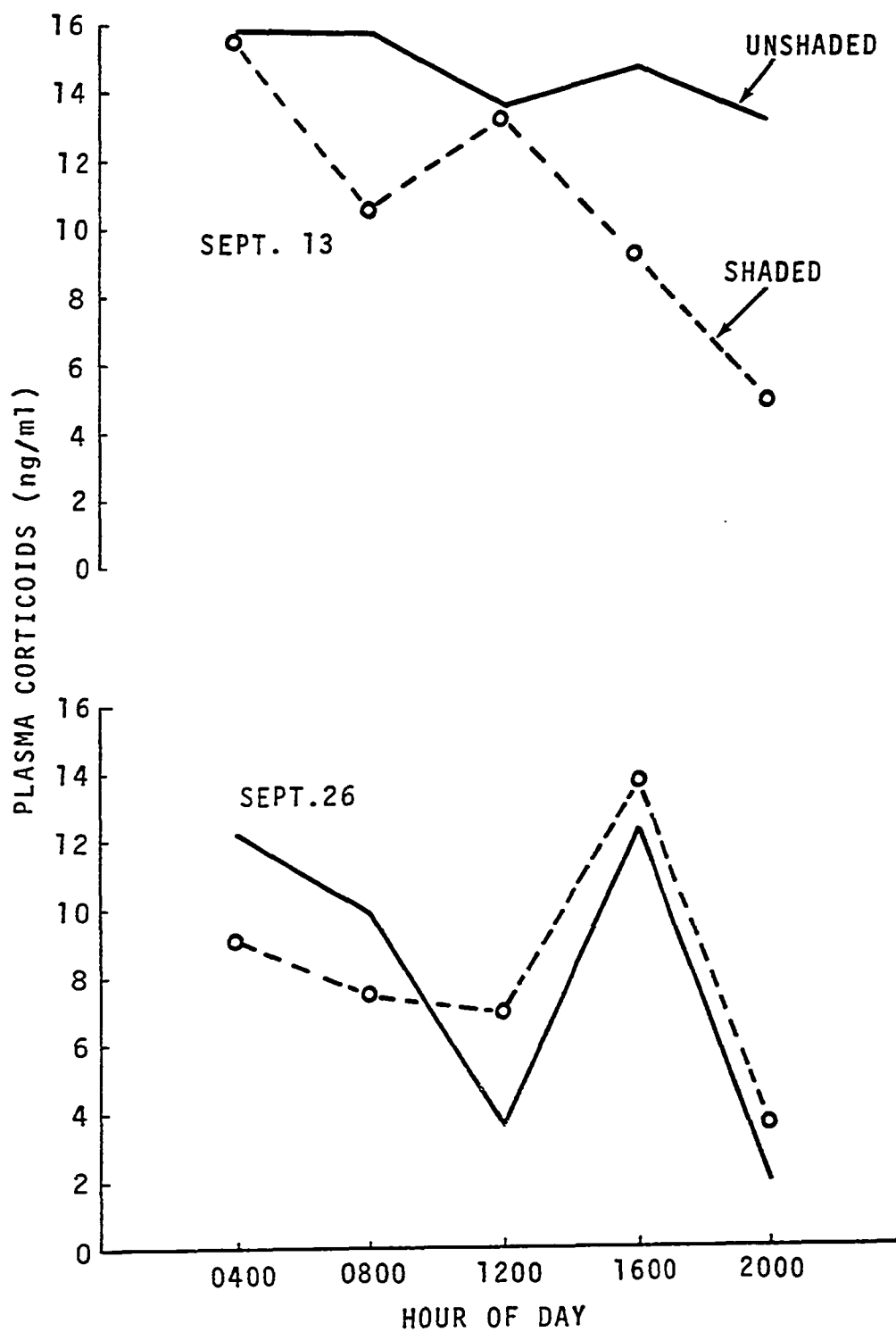




Figure 6. (Continued) Mean plasma corticoids at each bleeding by sample day for shaded and unshaded groups

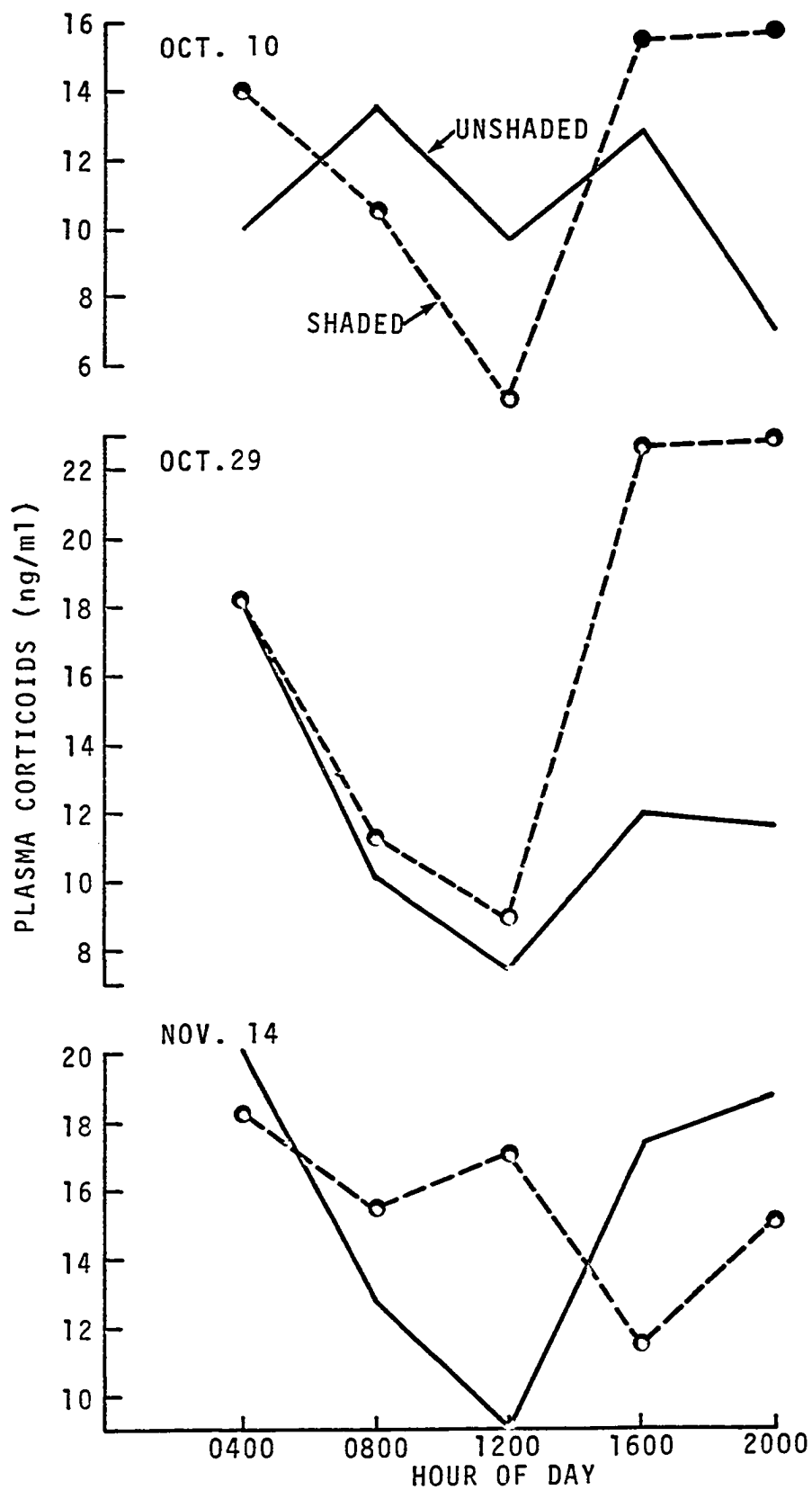
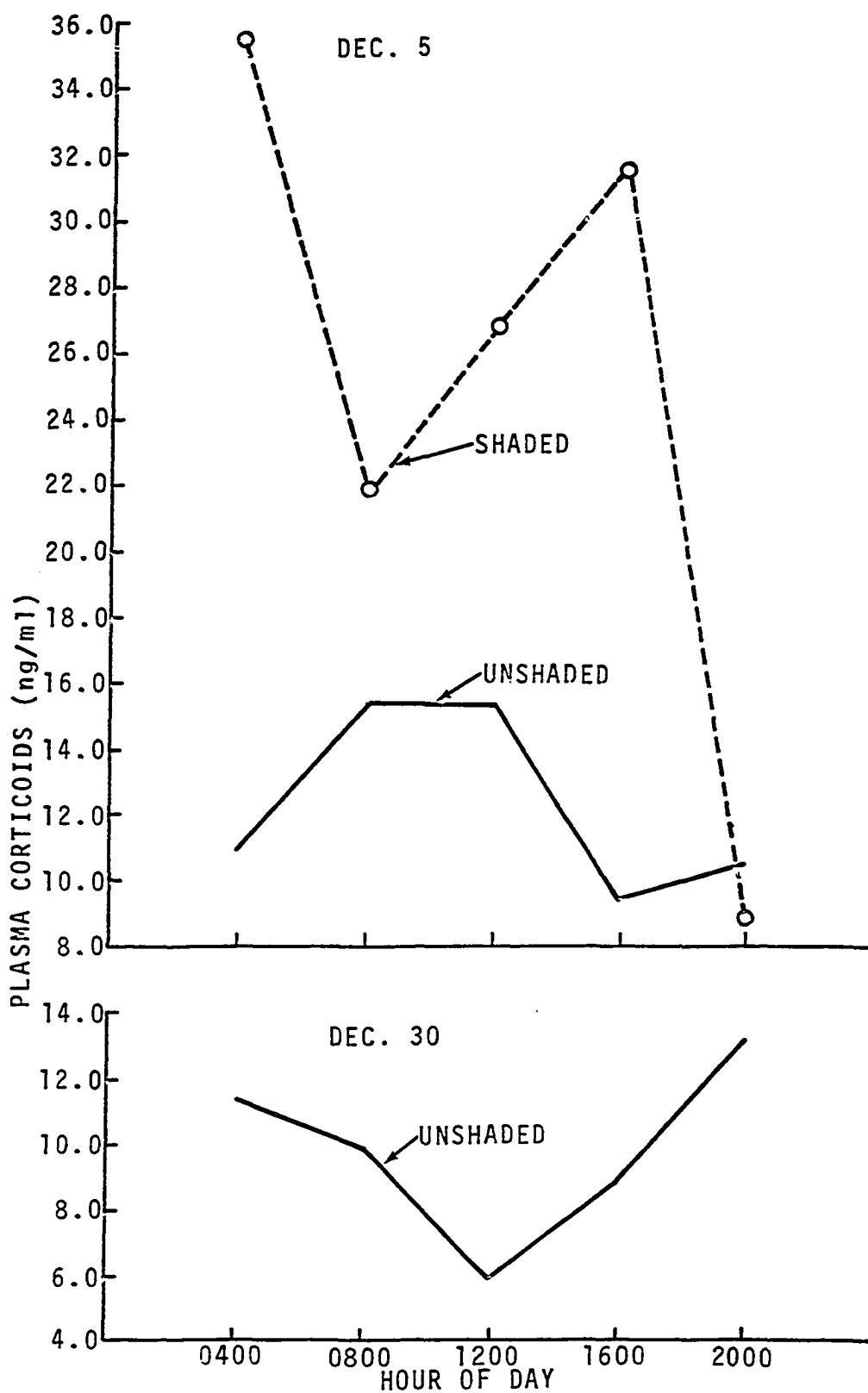


Figure 6. (Continued) Mean plasma corticoids at each bleeding by sample day for shaded and unshaded groups



### Plasma thyroxine

Plasma thyroxine was determined for 0400 and 1600 plasma samples for sample days from September 26 through December 5 using the Res-O-Mat ETR test (Appendix Table 108). There was a significant ( $r = 0.932$ ,  $P < .05$ ) trend for increased ETR values with time in unshaded cows as the season progressed and the weather became cooler (Figure 1c).

The THI at the time of bleeding and ETR were significantly correlated in unshaded animals at both 0400 ( $r = -.564$ ,  $df = 20$ ,  $P < .02$ ) and 1600 ( $r = -.626$ ,  $df = 19$ ,  $P < .005$ ). Shaded cows showed a significant ( $r = -.702$ ,  $df = 23$ ,  $P < .001$ ) relationship between THI and ETR only at 1600 (Figure 3c). Rectal temperatures were significantly ( $r = -.433$ ,  $df = 23$ ,  $P < .05$ ) correlated to ETR only in the shaded group and then only in the afternoon (Figure 4b). Plasma corticoids and ETR were not significantly correlated although there was a tendency for both to increase as the weather became cooler.

There was a significant ( $P < .002$ ) difference between 0400 and 1600 ETR values in the unshaded group on September 26 and October 10. There was a difference ( $P < .05$ ) in the shaded group only on September 26. The average THI on both of these days was 74.2 but September 26 had a higher THI (78) at 1600.

Table 108 in the Appendix gives a listing of ETR values by cow and sampling time.

### Milk production

The shaded group had a significantly ( $P < .001$ ) higher level of milk production than the unshaded group (Figure 1e). Mean production values for

the two groups were 18.5 and 14.5 kg of milk per cow per day, respectively. Values for milk, percentage milk fat and CMT scores are given in Appendix Table 110.

Results of a bivariate linear regression analysis (Tables 12 and 13) indicated that average THI (the mean of the daily average THI of the milk sampling day and the 2 preceding days) had a significant ( $P < .01$ ) effect on the milk production of the unshaded cows but not the shaded cows (Figure 8). The model, which also included time from the beginning of the experiment, accounted for 83% of the variance in average daily milk production in the shaded group and 85% in the unshaded group. However, since daily THI and daily advance in time were highly correlated in this study (see page 60), caution should be used in interpretation of these correlations. Milk production prediction formulas using THI and the number of days from the first sample (days) were

$$\hat{Y} = 46.348 - 0.0612 (\text{days}) - 0.385 (\text{THI}) \text{ for}$$

unshaded cows and

$$\hat{Y} = 43.865 - 0.0615 (\text{days}) - 0.304 (\text{THI}) \text{ for}$$

shaded cows. The predicted values are in kilograms of milk per cow per day.

The model predicts a 1.93 kg (10%) depression in daily milk for unshaded cows with an increase in THI from 70 to 75. The prediction was a 1.52 kg (6.8%) depression for shaded cows for the same THI change. Figure 8 presents predicted levels of production over the period of the experiment at several THI for shaded and unshaded cows. Rectal temperature and milk production were not significantly correlated in either group.

Table 12. Analysis of variance (linear model) for the influence of the daily advance of time (day) and average THI (3 days) on milk production in unshaded cows

| Source of Variance                       | df | SS     | MS     | F                 |
|--|----|--------|--------|-------------------|
| Regression due to both day and THI       | 2  | 34.013 | 17.007 | 43.4 <sup>a</sup> |
| Regression due to day, corrected for THI | 1  | 17.587 | 17.587 | 44.9 <sup>a</sup> |
| Regression due to THI, corrected for day | 1  | 3.859  | 3.859  | 9.8 <sup>b</sup>  |
| Residual                                 | 15 | 5.876  | .392   |                   |
| Total                                    | 17 | 39.889 |        |                   |

<sup>a</sup>(P<.0005)

<sup>b</sup>(P<.01)

Table 13. Analysis of variance (linear model) for the influence of the daily advance of time and average THI (3 days) on milk production in shaded cows

| Source of Variance                       | df | SS     | MS     | F                 |
|--|----|--------|--------|-------------------|
| Regression due to both day and THI       | 2  | 27.323 | 13.661 | 27.1 <sup>a</sup> |
| Regression due to day, corrected for THI | 1  | 13.724 | 13.724 | 27.2 <sup>a</sup> |
| Regression due to THI, corrected for day | 1  | 1.039  | 1.039  | 2.0               |
| Residual                                 | 11 | 5.538  | .503   |                   |
| Total                                    | 13 | 32.861 |        |                   |

<sup>a</sup>(P<.0005)

Shaded cows produced significantly ( $P < .001$ ) more milk fat (0.14 kg/cow/day) than unshaded cows. They also maintained a significantly ( $P < .001$ ) higher milk fat percentage up until October 29 at which time average THI began to decline below 74. At this time the trend reversed and unshaded cows tended to have higher milk fat percentages. There was a significant ( $r = -.781$ ,  $P < .005$ ) linear correlation between average THI and percentage milk fat at THI above 73 (Figure 9) in the unshaded group but not the shaded group.

California mastitis test (CMT) scores were significantly ( $P < .005$ ) higher in the unshaded group.

### Reproduction

Average values for rectal temperature, adrenal corticoid levels, ETR and milk are given by cow in Tables 14 and 15. Breeding data for each cow are also given. Two cows in the shade conceived and two unshaded cows conceived. Cow 648 was heat intolerant as can be noted by the average rectal temperature of 40 C. This cow was never observed in estrus. Rectal palpation of the ovaries indicated they were small and inactive.

Due to small numbers and wide variation in other parameters such as corticoid levels and ETR, evaluation of relationships between breeding performance and these data were not possible.



Figure 7. Milk production versus daily advance in time during the experiment for shaded and unshaded cows. THI was held constant at the level indicated

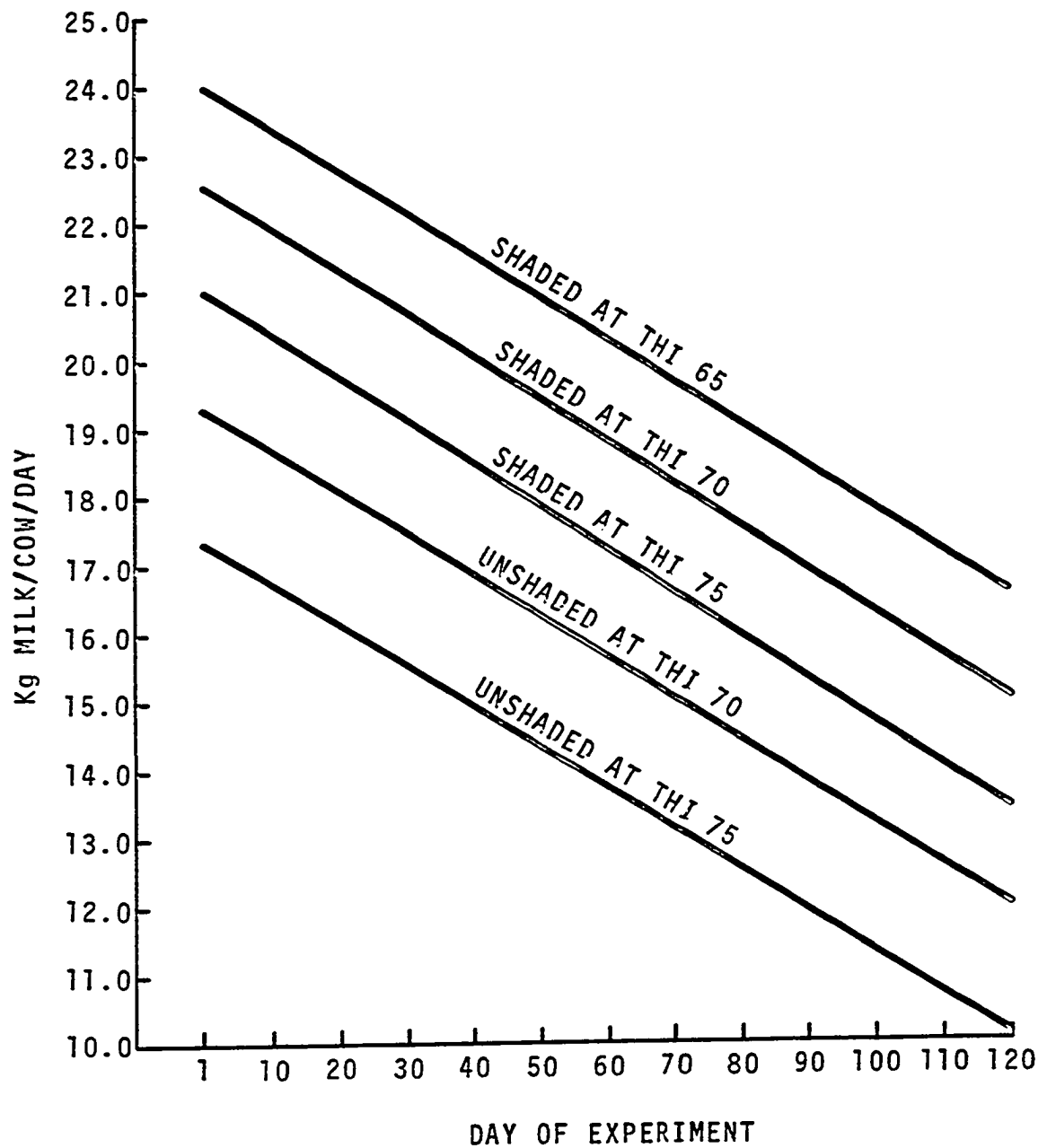


Figure 8. Daily milk production versus the average THI of 3 days ending with the milk sampling days for shaded and unshaded cows

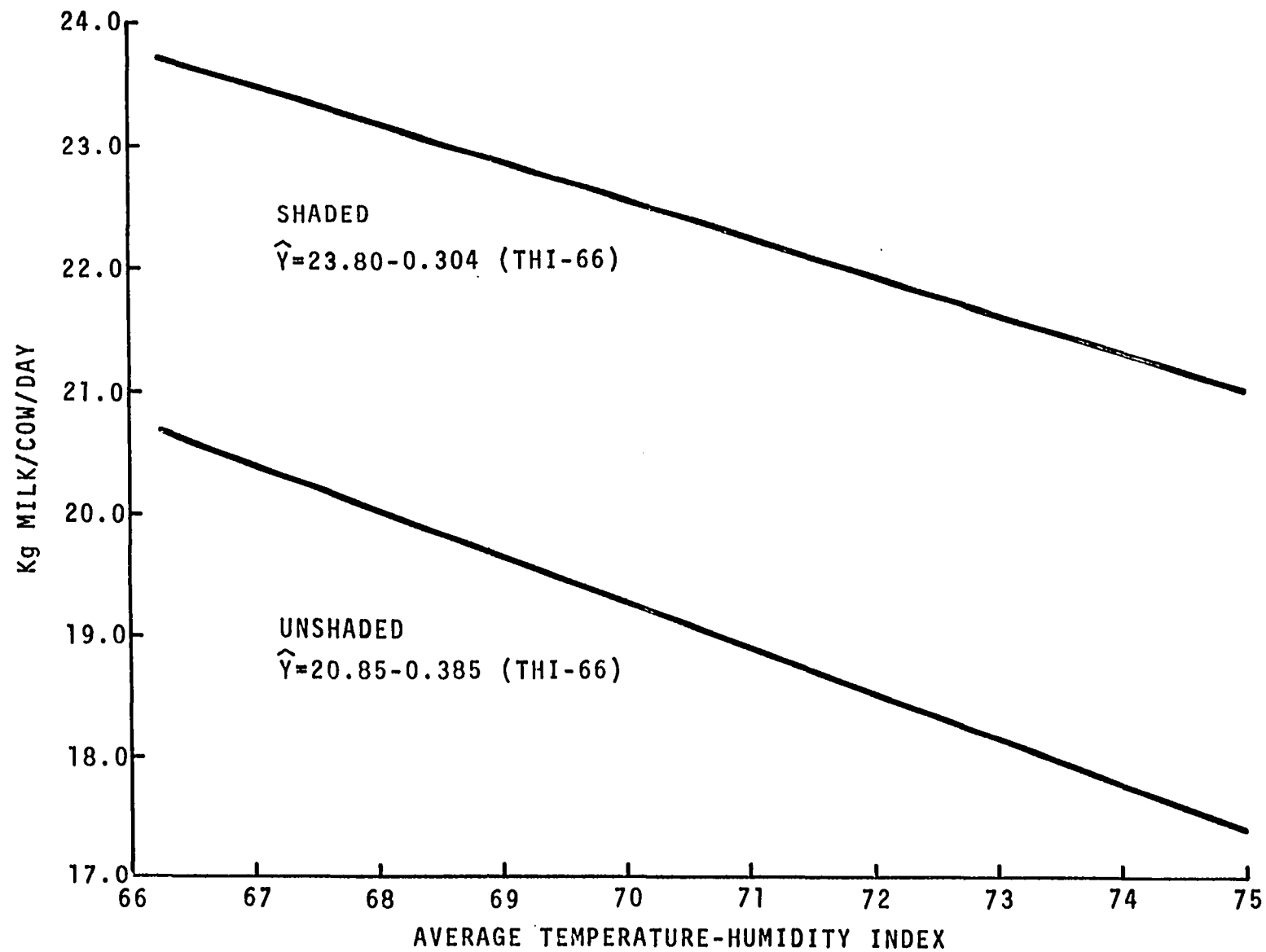
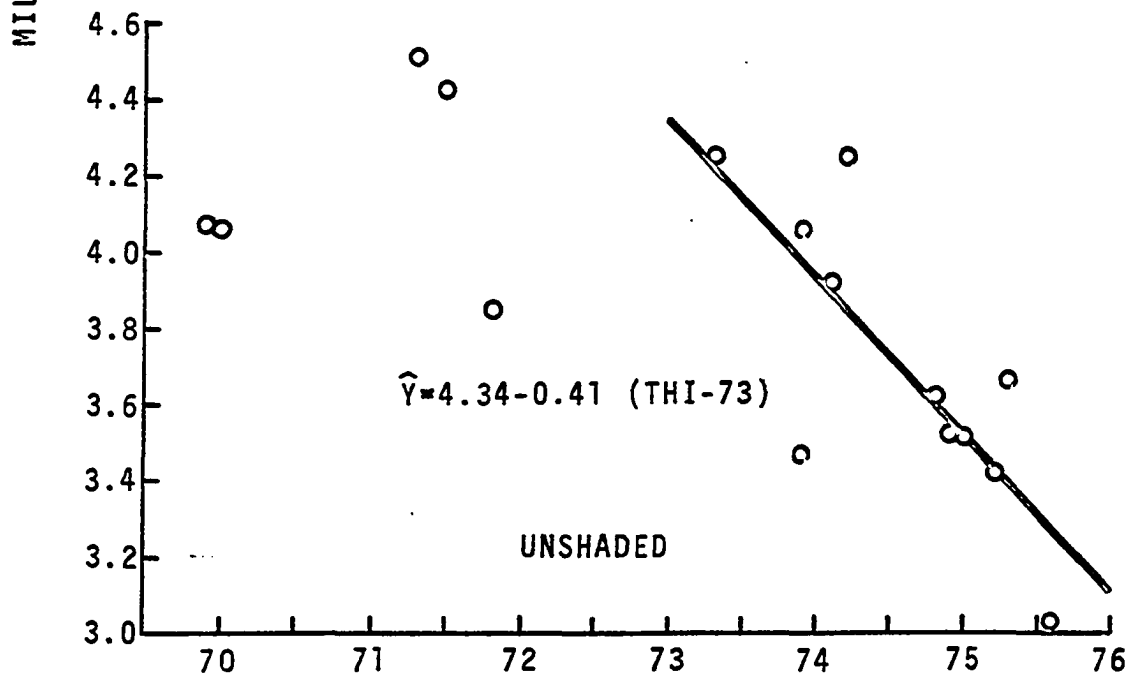
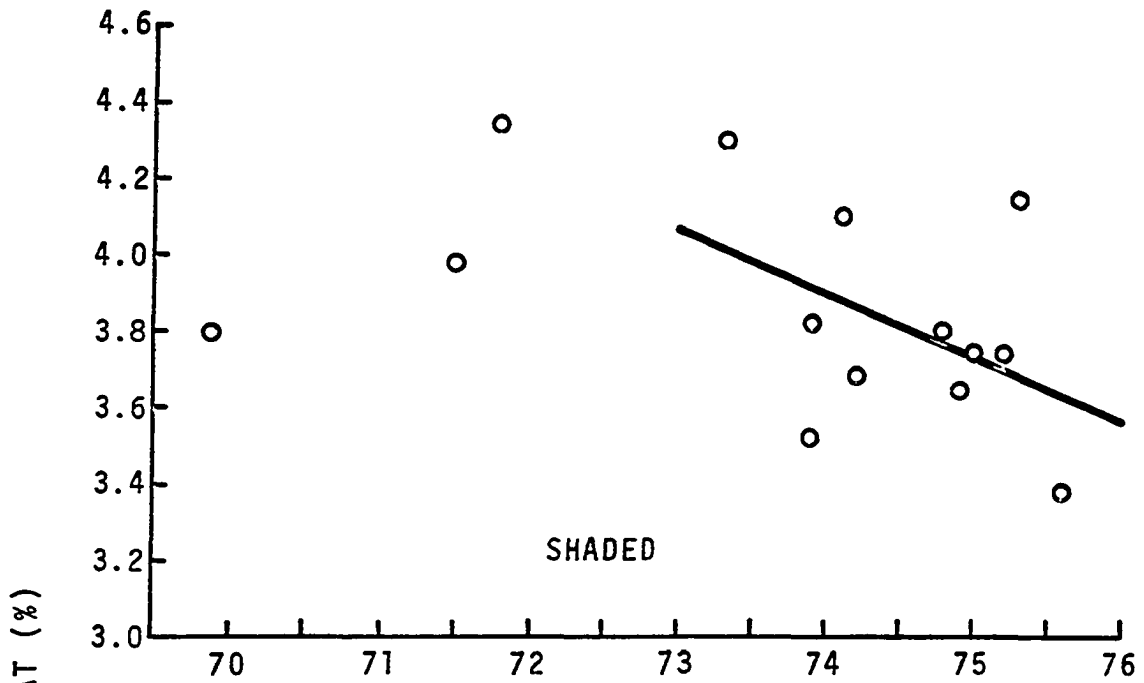


Figure 9. Percentage milk fat versus the average THI of the 3 days ending with the milk sampling day. The relationship was not significantly linear for the shaded group, but the least squares line is given for comparison with the unshaded group. For the unshaded group the relationship was linear at THI above 73 ( $r = -.781$ ,  $P < .005$ )



AVERAGE TEMPERATURE-HUMIDITY INDEX

Table 14. Summary of mean ( $\pm$ SD) values by cow of rectal temperature, plasma corticoids, ETR, milk and reproductive status for unshaded cows

|                               |   | Unshaded Cow Number           |                   |                   |                               | Mean              |
|-------------------------------|---|-------------------------------|-------------------|-------------------|-------------------------------|-------------------|
|                               |   | 550                           | 586               | 643               | 648                           |                   |
| Rectal Temp. (C)              |   | 39.29 $\pm$ 0.27 <sup>a</sup> | 39.21 $\pm$ 0.16  | 39.03 $\pm$ 0.24  | 40.00 $\pm$ 0.45 <sup>b</sup> | 39.38 $\pm$ 0.43  |
| Corticoid (ng/nl)             |   | 9.2 $\pm$ 4.9                 | 12.7 $\pm$ 4.8    | 16.6 $\pm$ 7.4    | 9.2 $\pm$ 2.5                 | 11.92 $\pm$ 3.5   |
| ETR                           |   | 0.904 $\pm$ 0.026             | 0.905 $\pm$ 0.043 | 0.922 $\pm$ 0.031 | 0.940 $\pm$ 0.044             | 0.918 $\pm$ 0.031 |
| Milk (kg)                     |   | 17.97 $\pm$ 3.47              | 11.01 $\pm$ 1.31  | 16.21 $\pm$ 1.10  | 12.86 $\pm$ 0.95              | 14.52 $\pm$ 3.15  |
| Calved                        |   | 7-15                          | 7-27              | 7- 4              | 8- 6                          |                   |
| Estrus                        | 1 | 9-16                          | 9-15              | 9-13              | ----                          |                   |
|                               | 2 | 10- 8                         | ----              | ----              | ----                          |                   |
|                               | 3 | 11- 4                         | ----              | ----              | ----                          |                   |
| Inseminated                   |   | 10- 9                         | 9-15              | 9-13              | ----                          |                   |
|                               |   | ----                          | ----              | ----              | ----                          |                   |
| Breeding Status<br>on Feb. 10 |   | NP <sup>b</sup>               | P <sup>c</sup>    | P                 | NP                            |                   |

<sup>a</sup>SD is between means of 6 sampling days.

<sup>b</sup>Non-pregnant.

<sup>c</sup>Pregnant.

Table 15. Summary of mean ( $\pm$ SD) values by cow of rectal temperature, plasma corticoids, ETR, milk and reproductive status for shaded cows

|                               |   | Shaded Cow Number             |                   |                   |                   |                   | Mean              |
|-------------------------------|---|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                               |   | 600                           | 608               | 633               | 637               | 646               |                   |
| Rectal Temp. (C)              |   | 39.04 $\pm$ 0.16 <sup>a</sup> | 38.85 $\pm$ 0.11  | 39.53 $\pm$ 0.27  | 39.22 $\pm$ 0.23  | 39.09 $\pm$ 0.25  | 39.15 $\pm$ 0.25  |
| Corticoid (ng/nl)             |   | 9.2 $\pm$ 6.0                 | 12.7 $\pm$ 7.3    | 14.4 $\pm$ 9.7    | 18.9 $\pm$ 6.7    | 20.4 $\pm$ 12.5   | 15.12 $\pm$ 4.5   |
| ETR                           |   | 0.897 $\pm$ 0.034             | 0.912 $\pm$ 0.037 | 0.902 $\pm$ 0.029 | 0.913 $\pm$ 0.020 | 0.954 $\pm$ 0.047 | 0.915 $\pm$ 0.025 |
| Milk (kg)                     |   | 20.99 $\pm$ 1.58              | 15.16 $\pm$ 2.96  | 18.82 $\pm$ 1.06  | 14.51 $\pm$ 1.88  | 23.07 $\pm$ 2.13  | 18.51 $\pm$ 1.31  |
| Calved                        |   | 7-20                          | 7-11              | 7-11              | 6-26              | 7-21              |                   |
| Estrus                        | 1 | 9-17                          | 9-10              | 9-19              | 9-25              | 9- 1              |                   |
|                               | 2 | -----                         | 10-24             | 10-12             | 11- 1             | 9-24              |                   |
|                               | 3 | -----                         | -----             | -----             | -----             | -----             |                   |
| Inseminated                   |   | 9-18                          | 9-10              | 9-20              | 9-25              | 9-25              |                   |
|                               |   | -----                         | 10-24             | 10-13             | -----             | -----             |                   |
| Breeding Status<br>on Feb. 10 |   | P <sup>b</sup>                | NP <sup>c</sup>   | NP                | NP                | P                 |                   |

<sup>a</sup>SD is between means of 6 sampling days.

<sup>b</sup>Pregnant.

<sup>c</sup>Non-pregnant.



## Discussion

The average daily THI of the majority of the days in this study would have been designated as mildly heat stressing (THI 71 to 74) under the guidelines suggested by Kibler (1964). Nevertheless, THI were correlated with rectal temperatures in unshaded cows ( $r = 0.880$ ,  $P < .01$ ), plasma corticoids in shaded cows ( $r = -.920$ ,  $P < .01$ ), plasma thyroxine in both shaded ( $r = -.916$ ,  $P < .05$ ) and unshaded ( $r = -.944$ ,  $P < .02$ ) cows and negatively correlated with milk production in unshaded ( $P < .01$ ) cows. Of particular interest was the response of the cows to the narrow diurnal THI flux which consisted of mild afternoon THI and relatively warm nights (Figure 2a).

Rectal temperatures of shaded cows at 1600 were not increased significantly over rectal temperatures at 0800 indicating that afternoon THI of themselves were not adding a significant heat load to these cows. In contrast, unshaded cows exhibited a significant ( $P < .01$ ) temperature rise (Figure 2b) in the afternoon indicating that the combination of THI and solar radiation was a major factor contributing to the heat stress of cows in this climate.

In addition to having elevated rectal temperatures during the day the cows failed to lose all of their excess heat at night. Rectal temperatures at 0800 (prior to the time that corrals were exposed to direct solar radiation in the morning) were above the 38.6 C considered normal on each of the sampling days. Although neither group cooled completely at night the unshaded group lost more heat and had lower rectal temperatures

at 0800 (Figure 2b), indicating an advantage in cooling if cows could radiate to the night sky. On some nights the cooling advantage may have come from shower activity and the evaporative cooling that it offered. Nonetheless, the slight advantage in heat loss at night for cows in the open dictates against completely covered loafing areas in this climate.

Warm nights and warm afternoons were coincidental during the experiment (Figure 2a) and, as might be expected, high rectal temperatures at 0800 were coincidental with high rectal temperatures at 1600 (Figure 2b). It appeared that rectal temperatures at 1600 were determined largely by a combination of the morning rectal temperature at 0800 and by afternoon THI. An analysis of variance for unshaded cows attributed 80% of the variability in rectal temperature at 1600 to these two factors with the larger effect being contributed by rectal temperature at 0800 (Table 8). Unfortunately solar radiation, which has been shown to be an important factor in the afternoon rectal temperature rise, was not part of this model. It was not included because solar radiation values for individual days were not available. THI and average weekly solar radiation are closely related ( $r = 0.93$ ,  $P < .01$ ). Since solar radiation has a causal relationship to THI because of its effect on temperature, it would be represented in the model. The same analysis for shaded cows attributed 49% of the variability of rectal temperatures at 1600 to rectal temperature at 0800. Average THI contributed essentially nothing to regression in this model (Table 9). It may be theorized from these results that efforts to cool cows at night might result in lower afternoon rectal temperatures especially if the cows had shade available. Cooling cows at night during

the warm season to ensure that they start the day with a normal heat balance needs further investigation especially since energy expenditure for cooling would potentially be least during this time of day.

Plasma adrenal corticoid levels were generally within the ranges reported by other workers (Wagner and Oxenreider, 1972) who used this method of assay. If the results are different, they are slightly elevated rather than depressed.

Mean plasma corticoid concentrations in the shaded and unshaded groups during the daily flux are compared in Figure 5. Although mean corticoid levels were higher ( $P < .05$ ) in the shaded group the variation between sampling days was large as can be seen in Figure 6. Aside from a tendency for corticoids to be depressed at mid-day and elevated before milking in one group or the other on each day, no single trend runs consistently through this series of graphs. Of particular interest is December 5 on which corticoid levels were substantially elevated (Figure 6) in the shaded group. The shaded cows were in pens adjacent to those of the unshaded cows and any general disturbance should have affected corticoid levels in both groups. However, on December 5 winds were gusting at about twice the normal speed. This was the first day since August with gusts in excess of 20 mph. The shades rattled more than usual and were actually damaged, which was one reason shaded versus unshaded comparisons were not carried beyond this date. This activity may have been enough to cause an increase in corticoid levels for cows under the shades. Since the point contributed by December 5 is largely responsible for the high correlation ( $r = -.920$ ,  $P < .01$ , Figure 3b) between THI and corticoids in

shaded cows it deserves special attention especially in light of the fact that corticoid levels in unshaded cows, which had been increasing along with those of the shaded cows as the weather cooled (Figure 1d), were decreased on that day and were still relatively low (10.1 ng/ml) on December 30, a day on which the cows were in good heat balance (average rectal temperature for the day in the unshaded group was 38.64 C). It may be that actual corticoid levels, which were not excessively low, were not the important issue here, but rather the relative ability of the heat stressed animals to respond with increased corticoid levels when a stimulus was applied. The tendency for shaded cows to have higher afternoon corticoid levels gives some support to this theory.

Further support for the thesis that heat stress was depressing plasma corticoid levels in some manner is given by the correlation between rectal temperature and corticoid levels at 0400 ( $r = -.384$ ,  $P < .02$ ) when data from the shaded and unshaded groups were pooled. However, this was the only sampling time when such a relationship was found for either group.

The increased premilking corticoid levels (0400 and 1600) seen in Figure 6 may have been related to anticipation of milking as suggested by Willet and Erb (1972) since cows were being removed from adjoining pens prior to and during sampling. Milk production and corticoid levels prior to milking were not linearly correlated in a bivariate analysis which considered time (days from the beginning of the experiment).

Plasma thyroxine was negatively correlated with THI (Figure 3c). A range of ETR values for the normal bovine animal is not available

presently. Therefore, the use of ETR in this experiment was intended to determine relative changes in plasma thyroxine rather than actual values. Refetoff et al. (1970) gave normal T4 and T3 uptake values for humans of 6.2 ug/100 ml and 30.2%, respectively. Values for European breeds of cattle were 5.3 ug/100 ml and 32.9%, respectively. These values would place the cow on the low side of normal human plasma thyroxine levels. Nevertheless, with the exception of five samples taken on September 26 (the first and most heat stressing day on which ETR were determined) all values were within the range (0.86 - 1.13) designated as euthyroid for human subjects. However, all but two values were below 1.00.

Values for ETR and THI were most highly correlated at 1600 in both the shaded and unshaded groups possibly indicating that the maximum heat stress of the day might set the level of thyroid function. On the more heat stressing days (September 26 and October 10) of the experiment the ETR values of cows in the unshaded group at 1600 were significantly depressed below ETR values at 0400. This occurred in the shaded group only on September 26 (Appendix Table 108). This afternoon depression in thyroid activity at THI above 74 coincided with a decline in milk fat percentage in unshaded cows which is shown in Figure 9. It appears that at this level of heat stress the animal introduced some mechanism for depressing plasma thyroxine during the hot part of the day that may have been affecting fat production. However, it is possible that the afternoon ETR depression may have been the result of increased plasma volume for cooling purposes rather than a real decline in plasma thyroxine. Whatever

the cause it appears to be part of the physiological change by which the animal adjusts to heat stress and deserves further investigation.

All milk production parameters favored the shaded cows. They produced significantly more milk and milk fat, maintained a higher fat test at THI above 74 and had lower CMT scores. These results were coincidental with lower afternoon rectal temperatures and higher plasma corticoid levels in this group. Average plasma thyroxine was essentially the same for both groups. Although increasing THI had a depressing effect on the milk production in the unshaded group, a relationship between milk production and plasma corticoid concentrations and plasma thyroxine was not established.

The experimental design used and the number of animals involved prohibit assignment of credit for increased production efficiency to the shade treatment. However, since the shaded cows were under less heat stress during the day as was indicated by their rectal temperatures, the shades undoubtedly deserve some credit.

Johnson et al. (1962) estimated a decline of 0.318 kg/cow/day for each unit increase in THI (in the range from 74 to 79) for cows producing between 13.4 and 18.2 kg of milk per day. This is reasonably close to the 0.304 and 0.385 kg/cow/day decline in milk predicted for the shaded and unshaded groups from the current study.

The mechanism through which heat stress depresses milk production requires some clarification. Johnson (1965a) postulated that environmental temperatures might affect milk production directly via (1) cutaneous receptors and the CNS by way of the hypothalamus and the pituitary,

(2) changes in the temperature of the blood perfusing the hypothalamic region and (3) temperature changes in the mammary gland, thereby altering the metabolic activity of the mammary tissue. He also suggested that milk production would be indirectly affected by the depressed nutrient intake that accompanies heat stress. With regard to the latter, McDowell et al. (1969) pointed out that milk energy output declined almost twice as much as digestible energy intake in the heat stressed cow. This would indicate that energy was being directed away from milk production, perhaps by means of one of the direct influences proposed above.

Maust et al. (1972) reported that in an average THI ( $\pm$ SD) of  $75 \pm 4.2$  both milk and milk fat were most closely related to weather conditions 3 days prior to sampling. They attributed this lag in milk production response to decreased feed intake prior to the sampling day. In a more heat stressing situation than theirs, a direct effect of heat stress was seen more clearly. Appendix Table 114 presents average rectal temperature, milk production and TDN consumption response of a group of 25 cows to THI between 77 and 81 (Ingraham, 1968). At these THI, milk and TDN consumption responded acutely to increased rectal temperature with little or no lag. During a 5-day period of naturally occurring heat stress, average rectal temperature increased 0.9 C (39.5 to 40.4 C). Milk production declined 3.7 kg while TDN consumption declined 1.3 kg. There was a linear relationship between the rectal temperature and the milk production and TDN intake of the same day ( $r = -.943$ ,  $P < .02$  and  $r = -.967$ ,  $P < .01$ , respectively). After average rectal temperature reached its peak it declined over the following 5 days. Rectal temperatures, milk production and TDN

were correlated during this period, although milk production did not return to its pre-stress level. These responses may indicate that the direct effect of heat stress on milk production becomes more pronounced at average THI above 77. Since the average THI of the current experiment were under 76 the physiologic changes which bring about this effect may not have been evident. That is, if thyroxine and the adrenal corticoids are involved, significant changes in concentration might not occur before THI rise above 76. This may be the critical THI at which the hypothalamus responds to external and internal stimuli by suppressing the production and/or the release of the releasing factors for the hormones which influence milk synthesis.

References concerning a direct effect of temperature in the mammary gland on milk synthesis are not readily available. Johnson (1965a) suggests that since the gland is peripherally located, milk synthesis may be influenced by high and low ambient temperature. It is possible that the gland is located outside of the body cavity because the enzyme systems involved in milk synthesis work most efficiently at temperatures lower than those in the body cavity. Fat synthesis may be an example, for fat appears to be preferentially layered under the skin, especially in the European breeds of cattle. This may be an indication that temperature can have an effect on the place that fat is synthesized in the body and might explain why milk fat synthesis is depressed in heat stress. In a consideration of the relationship of heat stress to decreased milk synthesis (fat) these points merit further careful investigation.



In addition to the indirect effect of reduced feed intake on milk production in heat stressed cattle, altered digestion in the rumen must also be considered. Most investigators have reported reduced ruminal volatile fatty acids with the principal reduction being in acetic acid. However, Stanley et al. (1971) evaluated high and low concentrate diets in heat stressed cattle (29.4 C, 85% RH) and reported finding no significant effect of heat stress on any measures of ruminal fatty acids when feed input was controlled. They concluded that some factor other than ruminal volatile fatty acid levels was responsible for the depressed milk fat that results when high concentrate diets are fed to heat stressed cows.

Mishra et al. (1970) reported that rumen pH was decreased in heat stressed cattle and suggested that methods of increasing ruminal pH should be investigated. Stanley et al. (1972) investigated the use of buffered concentrate rations in Hawaii in the herd used for the current experiment. They reported that 0.34 kg of  $\text{NaHCO}_3$  per day significantly increased ( $P < .05$ ) 4% fat corrected milk, daily fat production and acetic:propionic ratio. While this report is promising, it is evident that further investigations in the area of heat stress and rumen digestion will be necessary to resolve the confusion.

The California Mastitis Test (CMT) score gives an evaluation of the number of somatic cells in the milk. In the current experiment shaded cows had fewer milk somatic cells (better milk quality) than unshaded cows. Since CMT scores are not available for each animal before the experiment, caution should be exercised in evaluation of this result. A cross-over

experimental design in which each cow was tested in the sun and shade might have resolved this problem.

The effect of heat stress on milk somatic cell counts is a matter of current controversy. There is general agreement that cell counts increase during the hot season (Paape et al., 1973; Wegner and Stott, 1971). The controversy concerns whether the increased counts are the result of inflammation in the mammary gland or a simple response to heat stress. Paape et al. (1973) attempted to resolve this problem by heat stressing cows with no bacteria in their milk. Heat stress did not cause an increase in milk somatic cell counts of these cows. Wegner and Stott (1971) reported an increase in milk somatic cell count following injections of ACTH. This is in contrast to Paape et al. (1971) who also monitored the response of milk somatic cell counts to ACTH injections. Their results discounted the thesis that stress is a predisposing factor to abnormal somatic cell counts in non-infected quarters. However, their results did suggest that an increase in susceptibility to mastitis may result from stress because of a reduction in the phagocytic competence of the neutrophils in the cow's blood. Unfortunately, it will not be possible to interpret the significance of these experiments until more information is available concerning ACTH activity in heat stressed cattle.

Whatever the cause, milk somatic cell counts do increase during the warm season in many subtropical areas. In addition to a possible direct effect of heat stress, two factors probably contribute to the problem. More cows are in late lactation during the warm season in these areas as indicated below and daily weather changes may cause milk yields to be

erratic. Both factors predispose to overmilking if milkers are not alert and, consequently, may increase the incidence of irritated quarters.

Actual milk production losses for dairy herds during the warm season in the subtropics may be much larger than those predicted from increased THI. The additional milk loss is due to the inability of many dairies to maintain a stable milking herd during the summer months. That is, because of climate related breeding problems from June through October, fewer cows calve from April through August of the following year. Consequently, a higher proportion of the cows in the milking herd during the summer are in late lactation. The effect of this on herd milk production for a dairy in Mexico (see Experiment II, page 108) is presented in Appendix Table 112. For this dairy only about 50% of the hot season milk depression could be attributed directly to the effect of heat stress on the milk production of individual cows. The remainder was due to breeding problems, most of which were also the result of heat stress.

EXPERIMENT II: AN EVALUATION OF THE RELATIONSHIP BETWEEN  
THE AVERAGE TEMPERATURE-HUMIDITY INDEX OF DAYS DURING THE ESTROUS CYCLE  
AND CONCEPTION RATE WITH EMPHASIS ON THE TWO DAYS PRIOR TO INSEMINATION

Objectives

Although the reduced reproductive efficiency of heat stressed cattle is an established fact, comparatively little information is available to quantitate the effect of heat stressing climates on the conception rates of lactating cows. Data from controlled experiments are not available. However, data are available in the records of a few large, well-managed dairies in subtropical and tropical areas. Two such dairies, one in Mexico and the other in Hawaii, were the sources of the data used in this study which was proposed to determine the influence of adverse ambient temperature and humidity on conception rates. Specifically, answers to the following questions concerning conception rates (CR) and the temperature-humidity index (THI) were sought.

1. Over what THI range is CR depressed?
2. What is the nature of the CR versus THI response curve?
3. Do the CR versus THI response curves change with seasons and in succeeding years?
4. Are the CR versus THI response curves for these two dairies significantly different?
5. Can an effect of THI on CR, independent of seasonal effect, be demonstrated?
6. Is there a period prior to or following breeding when THI is more critical to CR?

7. If one period does affect CR more than another period, does the timing in relation to breeding give an indication of the point at which fertility is being depressed?

## Methods

### Herd environments

Mexico (MX) This dairy (600 Holstein cows) was established under good management in the latter part of 1962 near Culiacan on the west coast of Mexico. Feeding practices were good. Fresh-chopped alfalfa was available from February to June. Sorghum silage was fed when fresh alfalfa was not available. Alfalfa and sudan hay were also fed. The concentrate ration contained milo, rice bran, wheat bran, and cotton seed meal, and was fed according to recommended feeding standards.

Pens were relatively open to air movement, had sheet iron shades, and held 25 cows each. Cows were checked for estrus in the early morning and in the late afternoon by an individual responsible for this job. Milkers were also responsible for reporting cows in estrus.

Herd records for 1963, 1964 and 1965 were evaluated. In 1963 and 1964, all cows were hand bred by natural service by one of six bulls. In 1965, 55% of the cows were bred by natural service. The remainder were bred by artificial insemination using frozen semen from a supplier in Wisconsin. Breeding and production records were kept on individual cow cards. A hygrothermograph on the dairy provided ambient temperature and relative humidity data.

Milk production data for MX were evaluated in addition to breeding records. Daily total milk production to the nearest liter and the number of cows milking each day were recorded. The milk production of individual cows was weighed one day each month.

An experiment was conducted at MX during the summer of 1967. Daily milk production, feed consumption and rectal temperatures were recorded over a period of 20 days for 2 pens of 25 cows each. Rectal temperatures were taken with standard clinical thermometers prior to milking (0230 and 1330) while the cows were in their stanchions.

Hawaii (HW) This was a large (1300 Holstein cows), well-managed dairy on the north shore of Oahu, Hawaii, 7 miles east of the Waialeale Experimental Farm which was the location of Experiment I. Cows were kept in groups of 40 in unshaded pens that were open to air movement. Cows were fed by recommended standards on a high calorie, relatively low fiber diet consisting of a commercial concentrate mix, pineapple hay, pineapple silage and pineapple bran.

Checks for cows in estrus were conducted 3 times a day. Cows were bred by artificial insemination using semen supplied by American Breeders Service of DeForest, Wisconsin. Breeding and production records were kept on individual cow cards from which they were transcribed for this study.

Weather records, which include hygrothermograph charts and daily maximum and minimum ambient temperature, were supplied by a weather station 3 miles east of the dairy at Waialua. Data for 1965, 1967 and 1968 were evaluated.

In 1970, after a preliminary evaluation of the above data, a project was undertaken to determine the status of HW cows in the post-calving

period in terms of hemoglobin, PCV, plasma calcium and plasma phosphorus. Heparinized blood samples were obtained. Most samples were taken at 25 to 35 days post calving. Some cows were rebled at about 60 days post calving. Blood analyses were conducted by the Division of Animal Industry Veterinary Laboratory in Honolulu.

Hawaii (HL) Breeding records for 1968 and 1969 were obtained from two dairies (HL) on the leeward shore of Oahu. Each had approximately 600 Holstein cows. Weather data were obtained from hygrothermograph charts obtained from the weather station at Lualualei. The dairies were evaluated to determine the seasonal breeding pattern on this warmer side of the island. Breeding was by artificial insemination using the same source as HW. These cows were fed a diet similar to that used at HW but their level of input tended to be higher. Animals were in a higher state of body condition.

#### Definitions

The definitions of the following abbreviations are given to clarify their usage.

THI In this article THI indicates the average temperature-humidity index of a 24-hour period extending from midnight to midnight. It was calculated from dry bulb temperature (db, °F) and relative humidity (rh% ÷ 100) by the following formula:

$$THI = db - (0.55 - 0.55 rh) (db - 58).$$

For the dairy in Mexico it was calculated from hygrothermograph charts at 2-hour intervals with the 12 THI values averaged to give the average THI of the day. For the dairy in Hawaii average daily THI was

calculated from the average of the maximum and minimum ambient temperature and the average of their concurrent humidities. This latter method proved to give a reasonable estimate of average daily THI but gave a value that was  $0.57 \pm 0.48$  (mean difference  $\pm$  SD) THI units higher than the former methods. It should be noted that although an interval of midnight to midnight was used for calculating average THI in this evaluation, it may not be the best interval for all situations. For another dairy in Hawaii, which bred most of its cows earlier in the day, an average THI that was calculated from noon till noon showed higher correlations between THI and CR.

CR CR was determined by the delivery of a calf except for a few cows in the last three months of the study in Mexico. For these, pregnancy was determined by rectal palpation at 3 to 4 months post-breeding.

B B designates the day of breeding. Days before and after breeding are designated as B-n or B+n, respectively. These terms generally relate to the THI values of these various days, e.g., B-2<B-1 indicates that the THI for B-2 was less than the THI of B-1.

The relative effect of the THI of each of the days around breeding (B-2, B-1, B and B+1) on CR was determined by means of partial correlation coefficients.

#### Evaluation of data

The computer was utilized to sort breeding data into the THI class of days prior to and following breeding. For most of the evaluations the THI class was 2 THI units wide. Specifically, each THI class was delineated by even numbered digits. However, for evaluations involving



partial correlation coefficients each class was one THI unit wide. Graphing and linear correlation analysis were used to determine the relationship between THI and conception rate (CR). Individual THI were calculated to the nearest 0.1 unit. Therefore, for tests in which the THI of 2 succeeding days were compared, this was the smallest difference possible. Chi-square contingency tables were used to determine if differences in CR were significant between groups. Steel and Torrie (1960) was used as a statistical reference.

Intervals from calving to first service and from first service to second service were calculated for HW by year and by 2-month periods.

## Results

### Contrasts in herd environment

Monthly comparisons of the CR with THI for first service only and of all services are given in Figures 10 (MX), 11 (HW) and 12 (HL). Parallel studies with first service only were not significantly different from studies for all services. In view of this and to take advantage of the larger numbers in the all-service group, data from all services were used in these comparisons.

Although the dairies in this study were between latitudes 21°N and 25°N, they had contrasts in climate. MX was in the more heat stressing climate area. Fifty-three services occurred at THI above 84 for which the average CR was 13%. Mild, dry winters offered optimum breeding conditions. One hundred and twenty-five services occurred at THI under 64 during the winter of which 63% conceived. Periods of higher CR were coincident with

the availability of green alfalfa (February to June). However, CR decline began prior to discontinuing green alfalfa and improved prior to the time that alfalfa was available again thus reducing the possibility that feed factors were a major cause of CR fluctuations. Linear correlation coefficients for weather versus CR and milk production for 87 weekly periods at MX are given in Appendix Table 113. With the exception of maximum ambient temperature and relative humidity, all of the weather measures recorded (maximum, minimum and average ambient temperature, humidity and THI) were positively correlated ( $P < .001$ ). Average THI showed the highest correlation with CR ( $r = -.756$ ). The linear correlation coefficients for CR versus minimum ambient temperature and CR versus maximum ambient temperature were  $-.738$  ( $P < .001$ ) and  $-.565$  ( $P < .001$ ), respectively. The partial correlation coefficient for CR versus minimum ambient temperature with maximum ambient temperature held constant was  $-.575$  ( $P < .001$ ). For CR versus maximum ambient temperature with minimum ambient temperature held constant it was nonsignificant ( $-.074$ ).

Conception rates decreased with the onset of the rainy season in mid-June with its increased minimum temperatures and humidities. Tropical showers strong enough to cool the cows occurred only occasionally. However, many days had a trace of rain which only raised humidities. Air movement, which was usually under 5 mph during the summer, was often absent at night. CR versus THI response curves for MX showed a negative linear correlation for each of the 4 days studied around breeding (Table 16) and were reasonably repeatable in each of the 3 years studied (Table 17).

Conception rate evaluations for individual bulls or artificial insemination were not different from each other. Each showed about the same degree of fertility as the others in different seasons.

In contrast to MX, Hawaii has a rainy season that extends from October through April. The 10-year average monthly precipitation for these months in the HW area is 7.3 cm. This causes muddy corral conditions which places increased demands on management as well as giving an added environmental stress to the cow. The net result is that data from these months may be confounded by seasonal factors other than heat stress.

Hawaii (HW) has a mild warm season (Figure 11). Average daily THI were under 76 for 2 years of this study (1966 and 1967). The CR versus THI response curves at HW for these years were inconsistent (Table 17, Figure 13). In 1966, the average THI for July through October was 73 with a range of only 6 THI units. The response curve for this season in 1966 is shown in Figure 14. No relationship is shown between CR and THI. In 1967, the average THI for the same 4 months was 73.6. The year 1968 was more heat stressing with an average THI of 74.6 during these months. Twenty-three days had THI in excess of 76. This was the only year with a significant CR versus THI relationship and this relationship was significant only for B-2 out of all days tested (Table 18). Consequently, only 1968 data from HW were used in single day comparisons with MX. There were no apparent management changes in 1968, and the same inseminator serviced cows for all 3 years using semen from the same source.

The climate on the leeward side (HL) of Oahu is warmer and tends to have less air movement than the north shore where HW is located. Average

THI for HL during July through October in 1968 and 1969 were 76.3 and 76.4, respectively. This slight increase in THI compared to HW resulted in an accentuated seasonal CR flux for these dairies with CR of 15-25% in the warmer months (Figure 12).

#### The CR versus B-2 response curve

A negative linear relationship between CR and THI was apparent for both MX and HW. For MX it was especially apparent for B-2 (Figure 15, Table 16). In the THI range from 70 to 84, CR declined from 53% to 10%. The data for Figure 15 were split into two component seasons to give Figure 16. For one season (May 20 through September 20) the trend was toward increasingly heat-stressing weather. This response curve lies in THI ranges above 70 and is highly linear ( $r = -.993$ ,  $P < .001$ ). The other response curve, for which the seasonal trend is toward cooler weather (September 21 through January 20), is linear above THI 74 but changes slope below THI 74. In Figure 16, it should be noted that in the season in which THI were decreasing (fall and winter) CR were significantly ( $P < .025$ ) higher in each THI range above 74 than in the season when THI were increasing (spring and summer).

The CR versus B-2 response curves for HW (Figures 17 and 18) are similar in many respects to those at MX. That is, the curve for the full year tends to be linear ( $r = -.988$ ,  $P < .02$ , Figure 17) above THI 66 and the warm season component of the curve remains highly linear ( $r = -.988$ ,  $P < .02$ , Figure 18) above THI 70. Conception rates at each THI were also improved in the warm season curve over those in the earlier season. Conception rate

versus THI prediction formulas for response curves from the warm season at MX and HW are  $CR = 62.3 - 4.2 (THI - 70.0)$  and  $CR = 66.2 - 4.7 (THI - 70.0)$ , respectively.

#### Influence of B-2, B-1 and B on CR

The importance of B-2 with respect to CR      The THI of 4 days (B-2, B-1, B and B+1) were evaluated as to their contribution to CR by means of partial correlation coefficients. That is, the THI of each day was evaluated with the effect of the THI of each of the other days held constant. The objective was to determine the relative influence of the THI of each individual day on CR. The partial correlation coefficients (Table 16) indicate that B-2 had significantly more effect on CR than any of the other 3 days at both HW ( $P < .002$ ) and MX ( $P < .02$ ).

Influence of B-2 on the CR versus B-1 response curve      An indication of the effect of B-2 on the B-1 response curve can be seen in Figures 19 (MX) and 20 (HW). In both of these figures data for the first day prior to the day of breeding were split into two component groups, (1) for the case in which B-2 was lower than B-1 and (2) for the case in which B-2 was higher than B-1. The two resulting component response curves are graphed in the aforementioned figures. At both dairies CR were significantly ( $P < .001$ ) higher if B-2 fell in the first group ( $B-2 < B-1$ ) than if it fell in the second group ( $B-2 > B-1$ ). Chi-square statistical evaluations are given in Tables 19 (MX) and 20 (HW). The results indicate that the probability of conception was significantly increased if the second day prior to breeding had a lower THI than the day prior to breeding. In

order to determine if the depression of CR in  $B-2 > B-1$  was predominantly a one-season phenomenon the data for Figures 19 and 20 were split into two component seasons. The first included the inseminations which occurred during months of the year when THI was increasing and the second included months when THI was decreasing. The resulting graphs are given in Figure 21. The response curve for  $B-2 > B-1$  was depressed in both seasons on both dairies indicating that the CR depression was not the result of something occurring in just one season.

Influence of  $B-1$  on the CR versus  $B$  response curve at MX At MX (but not HW) the same kind of relationship that was discussed above also existed between  $B-1$  and  $B$  (Figure 22). That is, the probability of conception was significantly increased if  $B-1$  was lower than  $B$  as compared to the case in which  $B-1$  was higher (Table 19). Figure 23 presents the case in which  $B-2 < B-1 < B$  is compared to  $B-2 > B-1 > B$ .

The response curves in Figure 23 are erratic, at least in part, because the number of services at each point has been reduced. A slightly different approach to the data has been used in Figure 24. In this case, the THI of the 2 days prior to breeding have been averaged so that no data were eliminated. In the THI range from 68 to 82, conception rates were significantly (10.4%,  $P < .0005$ ) improved if the average THI of the 2 days prior to the day of breeding were lower than on the day of breeding.

An examination of the data for the CR versus  $B$  response curve at MX revealed that the  $[(B-2) + (B-1)]/2 > B$  component was not significantly depressed if the difference in THI between the average and  $B$  was less than 0.8 THI units. As the difference increased, the CR depression became

larger. For example, in the THI range between 68 and 79, if the difference between the average and B was 0.8 to 2.2 THI units there was an average CR difference of 13.3% ( $P < .025$ ). If the THI difference was over 2.2 units there was an average CR difference of 19.3% ( $P < .025$ ).

Influence of B on the CR versus B+1 response curve at HW Although the effect of B-1 on the response curve of B for HW was not significant, there was a significant ( $P < .001$ ) effect of B on the B+1 response curve (Figure 25, Table 20). As with  $B-2 < B-1$ , in this case if  $B < B+1$ , there appears to be little or no relationship between CR and THI. On the other hand, CR is depressed (9.7%) for  $B > B+1$ . That is, the probability of conception was significantly better if the day of breeding had a lower THI than B+1 in the THI ranges above 68. At MX, CR was also higher if  $B < B+1$  but the difference was not significant. The combined effect of  $B-2 > B-1$  with  $B > B+1$  at HW is seen in Figure 26 and its significance given in Table 20. If both B-1 and B+1 were preceded by days with higher THI, CR was depressed 17.8%.

CR versus B-16 to B+2 inclusive at HW

A linear correlation analysis was computed to determine the relationship between CR and the THI of each of 16 days prior to breeding and 2 days following breeding from warm season data for the 3 years at HW (Tables 21 and 22). The response curves for 3 days (B-2, B-10 and B-14) are graphed in Figure 27. The curve for B-14 indicates that there was no relationship between the THI of that day and CR but the curve for B-10 shows a high degree of correlation ( $r = -.994$ ,  $P < .001$ ) with CR. The curve

for B-2 is also highly significant ( $r = -.978$ ,  $P < .005$ ). The slope of B-10 is greater ( $P < .001$ ) than the slope for B-14 and the slope of B-2 is greater ( $P < .005$ ) than the slope for B-10.

Since the slope of the response curve can be considered an index of the sensitivity of CR to increased THI, the slopes of the above response curves were plotted to determine whether the THI of some period during the estrous cycle might be relatively more damaging to CR (Figure 28). There was a significant ( $r = 0.878$ ,  $P < .001$ ) increase in slope with time from the fourteenth day prior to breeding to the second day prior to breeding. However, there is a depression in the graph for days 7, 8 and 9 prior to breeding. The second and third days prior to breeding were most sensitive to increased THI. The slope for CR versus B was significantly ( $P < .05$ ) less than the slope for the CR versus B-2 response curve.

#### Interval to first service and between services for HW

The interval from calving to first service was computed by 2-month periods for each year of the HW study (Appendix Table 116). This interval tended to be shortest (82 days) for cows calving in the warm season and longest (90 days) for cows calving in the winter months, when muddy corral conditions prevailed and the breeding program did not always get priority.

The average interval between first and second service was shortest (42 days) from March through June, longer (51 days) from July through October, and longest (60 days) in November and December (Appendix Table 117).

Intervals between first and second service and second and third ser-



vice that were between 15 and 50 days in length were pooled and graphed for Figure 29. Significantly ( $\chi^2 = 6.08$ ,  $P < .025$ ) fewer intervals were of 18 to 24 days duration during the period from July 1 through October than in the period from March 1 through June (31 vs 38% of the total intervals). On the other hand, a significantly ( $\chi^2 = 7.41$ ,  $P < .025$ ) higher percentage of the intervals were of 25 to 34 days duration during the July to October period than during the March to June period (10 vs. 6% of the total intervals).

#### Selected post-calving blood values at HW

During 1970, blood samples were taken from a representative group of HW cows to survey the possibility of certain nutritional deficiencies in this herd during the post-calving period. Average ( $\pm$ SD) plasma calcium and phosphorus for 60 cows were  $9.47 \pm 0.48$  mg/100 ml and  $5.38 \pm 0.89$  mg/100 ml, respectively. Hemoglobin values declined during the first 30 days of lactation. Forty-four cows between 25 and 35 days postpartum had an average hemoglobin of  $9.86 \pm 1.06$  g/100 ml. Nineteen cows bled between 25 and 35 days postpartum and rebled 28 days later had first and second sample values of  $9.80 \pm 1.04$  and  $11.47 \pm 1.20$  g/100 ml, respectively. Twenty-eight samples from animals 2 to 31 days postpartum (average  $17.2 \pm 10.1$  days) showed a linear decline ( $r = -.494$ ,  $P < .01$ ) in hemoglobin with days in lactation.

Table 16. Linear correlation information for conception rate versus B-2, B-1, B and B+1 for MX and HW

|           | Y Intercept<br>THI 65 | Slope per<br>THI Unit | Linear<br>Correlation<br>Coefficient | Partial<br>Correlation<br>Coefficient |
|-----------|-----------------------|-----------------------|--------------------------------------|---------------------------------------|
| <u>MX</u> |                       |                       |                                      |                                       |
| B-2       | 65                    | -2.8                  | -0.925 <sup>a</sup>                  | -0.595 <sup>b</sup>                   |
| B-1       | 62                    | -2.4                  | -0.915 <sup>a</sup>                  | -0.112                                |
| B         | 64                    | -2.6                  | -0.903 <sup>a</sup>                  | -0.238                                |
| B+1       | 63                    | -2.5                  | -0.922 <sup>a</sup>                  | +0.077                                |
| <u>HW</u> |                       |                       |                                      |                                       |
| B-2       | 63                    | -2.3                  | -0.925 <sup>a</sup>                  | -0.864 <sup>a</sup>                   |
| B-1       | 54                    | -0.8                  | -0.574 <sup>c</sup>                  | -0.289                                |
| B         | 53                    | -1.1                  | -0.705 <sup>b</sup>                  | +0.280                                |
| B+1       | 50                    | -0.4                  | -0.368                               | -0.468                                |

<sup>a</sup>(P<.002).

<sup>b</sup>(P<.02).

<sup>c</sup>(P<.05).

Table 17. Linear correlation information for average monthly conception rate versus the average THI for each month by year at MX and HW. Only months with THI of 70 or higher were used

| Year                 | Y Intercept<br>at THI 70 | Slope per<br>THI Unit | Correlation<br>Coefficient | Number of<br>Months |
|----------------------|--------------------------|-----------------------|----------------------------|---------------------|
| <u>MX</u>            |                          |                       |                            |                     |
| 1963                 | 62                       | -4.1                  | -0.947 <sup>a</sup>        | 9                   |
| 1964                 | 64                       | -3.8                  | -0.987 <sup>a</sup>        | 7                   |
| 1965                 | 56                       | -3.1                  | -0.940 <sup>a</sup>        | 9                   |
| Average <sup>b</sup> | 61                       | -3.7                  | -0.945 <sup>a</sup>        | 25                  |
| <u>HW</u>            |                          |                       |                            |                     |
| 1966                 | 69                       | -5.0                  | -0.606                     | 7                   |
| 1967                 | 52                       | +0.4                  | -0.160                     | 8                   |
| 1968                 | 48                       | -1.0                  | -0.242                     | 8                   |
| Average <sup>b</sup> | 59                       | -2.6                  | -0.423 <sup>c</sup>        | 23                  |

<sup>a</sup>(P<.001).

<sup>b</sup>Least squares line calculated with all points given by individual years.

<sup>c</sup>(P<.05).

Table 18. Linear correlation information for conception rate versus the THI of the second day prior to the day of breeding (B-2) by year at MX and HW. Data for THI values of 70 and higher were included for MX while values of 66 and higher were used for HW

| Year                 | Y Intercept<br>at THI 70 | Slope per<br>THI Unit | Correlation<br>Coefficient | Number of<br>Points |
|----------------------|--------------------------|-----------------------|----------------------------|---------------------|
| <u>MX</u>            |                          |                       |                            |                     |
| 1963                 | 68                       | -4.7                  | -0.969 <sup>a</sup>        | 7                   |
| 1964                 | 58                       | -3.1                  | -0.943 <sup>a</sup>        | 7                   |
| 1965                 | 52                       | -2.8                  | -0.970 <sup>a</sup>        | 7                   |
| Average <sup>b</sup> | 58                       | -3.6                  | -0.995 <sup>a</sup>        | 7                   |
| <u>HW</u>            |                          |                       |                            |                     |
| 1966                 | 56                       | 0.6                   | 0.657                      | 5                   |
| 1967                 | 51                       | 0.1                   | 0.175                      | 5                   |
| 1968                 | 52                       | -2.2                  | -0.975 <sup>a</sup>        | 6                   |
| Average <sup>b</sup> | 51                       | -1.7                  | -0.964 <sup>a</sup>        | 6                   |

<sup>a</sup>(P<.005).

<sup>b</sup>Data for the 3 years were pooled and a regression line calculated.

Table 19. The number of cows serviced, conception rate and chi-square values for differences in conception rates caused by the THI of 2 days before breeding at MX

| Days        | Cows Serviced | % Conception | Chi-Square          | THI Range |
|-------------|---------------|--------------|---------------------|-----------|
| B-2<B-1     | 412           | 37.1         |                     |           |
| B-2>B-1     | 350           | 25.1         |                     |           |
| Differences | 62            | 12.0         | 12.587 <sup>a</sup> | 72-82     |
| B-1<B       | 755           | 38.4         |                     |           |
| B-1>B       | 596           | 30.4         |                     |           |
| Differences | 159           | 8.0          | 9.484 <sup>a</sup>  | 68-84     |
| B-2<B-1<B   | 407           | 40.8         |                     |           |
| B-2>B-1>B   | 273           | 28.9         |                     |           |
| Differences | 128           | 11.9         | 9.956 <sup>a</sup>  | 68-84     |

<sup>a</sup>(P<.001).

Table 20. The number of cows serviced, conception rate and chi-square values for differences in conception rates caused by the THI of some days before and after breeding at HW

| Days           | Cows Serviced | % Conception | Chi-Square          | THI Range |
|----------------|---------------|--------------|---------------------|-----------|
| B-2<B-1        | 858           | 50.2         |                     |           |
| B-2>B-1        | 685           | 41.5         |                     |           |
| Difference     | 173           | 8.7          | 11.790 <sup>a</sup> | 68-78     |
| B<B+1          | 867           | 51.2         |                     |           |
| B>B+1          | 684           | 41.5         |                     |           |
| Difference     | 183           | 9.7          | 14.461 <sup>a</sup> | 68-78     |
| B-2<B-1, B<B+1 | 459           | 54.5         |                     |           |
| B-2>B-1, B>B+1 | 317           | 36.7         |                     |           |
| Difference     | 142           | 17.8         | 24.036 <sup>a</sup> | 68-78     |

<sup>a</sup>(P<.001).

Table 21. The number of cows inseminated (NC) and percentage conception in each THI range for each day from day 16 prior to breeding to the second day following breeding at HW. Percentage conception was rounded to calculate the linear correlation data given in Table 22

|      | THI Class Center |      |     |      |      |      |     |      |     |      |
|------|------------------|------|-----|------|------|------|-----|------|-----|------|
|      | 69               |      | 71  |      | 73   |      | 75  |      | 77  |      |
| Day  | NC               | %    | NC  | %    | NC   | %    | NC  | %    | NC  | %    |
| B-16 | 118              | 52.5 | 345 | 57.9 | 1044 | 50.0 | 670 | 47.4 | 85  | 49.4 |
| B-15 | 121              | 54.5 | 397 | 55.9 | 1055 | 49.1 | 589 | 47.7 | 106 | 53.7 |
| B-14 | 126              | 55.5 | 379 | 49.6 | 1028 | 51.6 | 638 | 47.6 | 102 | 53.9 |
| B-13 | 124              | 51.6 | 382 | 51.0 | 993  | 51.4 | 651 | 49.6 | 113 | 38.9 |
| B-12 | 112              | 59.8 | 375 | 50.6 | 1061 | 50.3 | 615 | 49.5 | 111 | 48.6 |
| B-11 | 112              | 58.9 | 395 | 52.6 | 1026 | 52.8 | 665 | 46.6 | 100 | 40.0 |
| B-10 | 121              | 58.6 | 384 | 54.6 | 1045 | 51.4 | 648 | 46.4 | 112 | 43.7 |
| B- 9 | 110              | 54.5 | 376 | 53.7 | 1089 | 51.2 | 661 | 47.8 | 88  | 43.1 |
| B- 8 | 111              | 59.4 | 374 | 54.5 | 1102 | 49.9 | 632 | 49.2 | 101 | 45.5 |
| B- 7 | 128              | 60.9 | 397 | 53.1 | 1062 | 49.9 | 649 | 48.0 | 100 | 50.0 |
| B- 6 | 106              | 65.0 | 394 | 54.3 | 1104 | 50.6 | 646 | 47.9 | 87  | 39.0 |
| B- 5 | 102              | 66.6 | 380 | 51.8 | 1123 | 50.0 | 643 | 50.0 | 98  | 39.7 |
| B- 4 | 105              | 63.8 | 405 | 51.8 | 1072 | 50.8 | 671 | 48.7 | 91  | 41.7 |
| B- 3 | 105              | 65.7 | 433 | 53.3 | 1050 | 51.6 | 652 | 47.2 | 110 | 34.5 |
| B- 2 | 106              | 62.2 | 389 | 55.2 | 1126 | 52.6 | 625 | 44.9 | 106 | 34.9 |
| B- 1 | 94               | 67.0 | 408 | 53.6 | 1096 | 50.8 | 640 | 47.0 | 110 | 49.0 |
| B- 0 | 105              | 60.9 | 421 | 52.7 | 1125 | 51.6 | 616 | 47.4 | 117 | 41.8 |
| B+ 1 | 79               | 60.7 | 387 | 51.9 | 1170 | 50.5 | 649 | 51.3 | 106 | 40.5 |
| B+ 2 | 72               | 55.5 | 420 | 52.6 | 1179 | 52.1 | 609 | 47.2 | 108 | 48.1 |

Table 22. Linear correlation information for CR versus the THI of each of the 16 days prior to the day of breeding and 2 days following breeding for HW. Data include 2350 services which occurred from April through August in 1966, 1967 and 1968 at THI between 68 and 78

| Day  | Intercept <sup>a</sup> | Slope | Correlation Coefficient | Significance |
|------|------------------------|-------|-------------------------|--------------|
| B-16 | 55.4                   | -0.85 | -.639                   | NS           |
| B-15 | 54.2                   | -0.40 | -.362                   | NS           |
| B-14 | 52.8                   | -0.20 | -.221                   | NS           |
| B-13 | 55.3                   | -1.35 | -.789                   | NS           |
| B-12 | 57.9                   | -1.15 | -.819                   | 0.1          |
| B-11 | 61.4                   | -2.20 | -.967                   | 0.01         |
| B-10 | 60.7                   | -1.95 | -.994                   | 0.001        |
| B- 9 | 57.0                   | -1.40 | -.955                   | 0.02         |
| B- 8 | 60.1                   | -1.70 | -.985                   | 0.005        |
| B- 7 | 59.1                   | -1.35 | -.832                   | 0.05         |
| B- 6 | 65.9                   | -2.90 | -.970                   | 0.01         |
| B- 5 | 65.8                   | -2.80 | -.912                   | 0.05         |
| B- 4 | 63.3                   | -2.35 | -.934                   | 0.02         |
| B- 3 | 67.6                   | -3.40 | -.961                   | 0.01         |
| B- 2 | 66.0                   | -3.20 | -.978                   | 0.005        |
| B- 1 | 64.3                   | -2.15 | -.858                   | 0.100        |
| B- 0 | 62.2                   | -2.20 | -.979                   | 0.005        |
| B+ 1 | 61.5                   | -2.15 | -.911                   | 0.05         |
| B+ 2 | 56.0                   | -1.00 | -.933                   | 0.05         |

<sup>a</sup>Intercept at THI 68.



Figure 10. Average monthly conception rates and average THI ( $\pm$ SD) by 15-day periods for MX in 1964 and 1965. A similar graph presenting maximum and minimum temperatures and humidity is given in Appendix Table 111

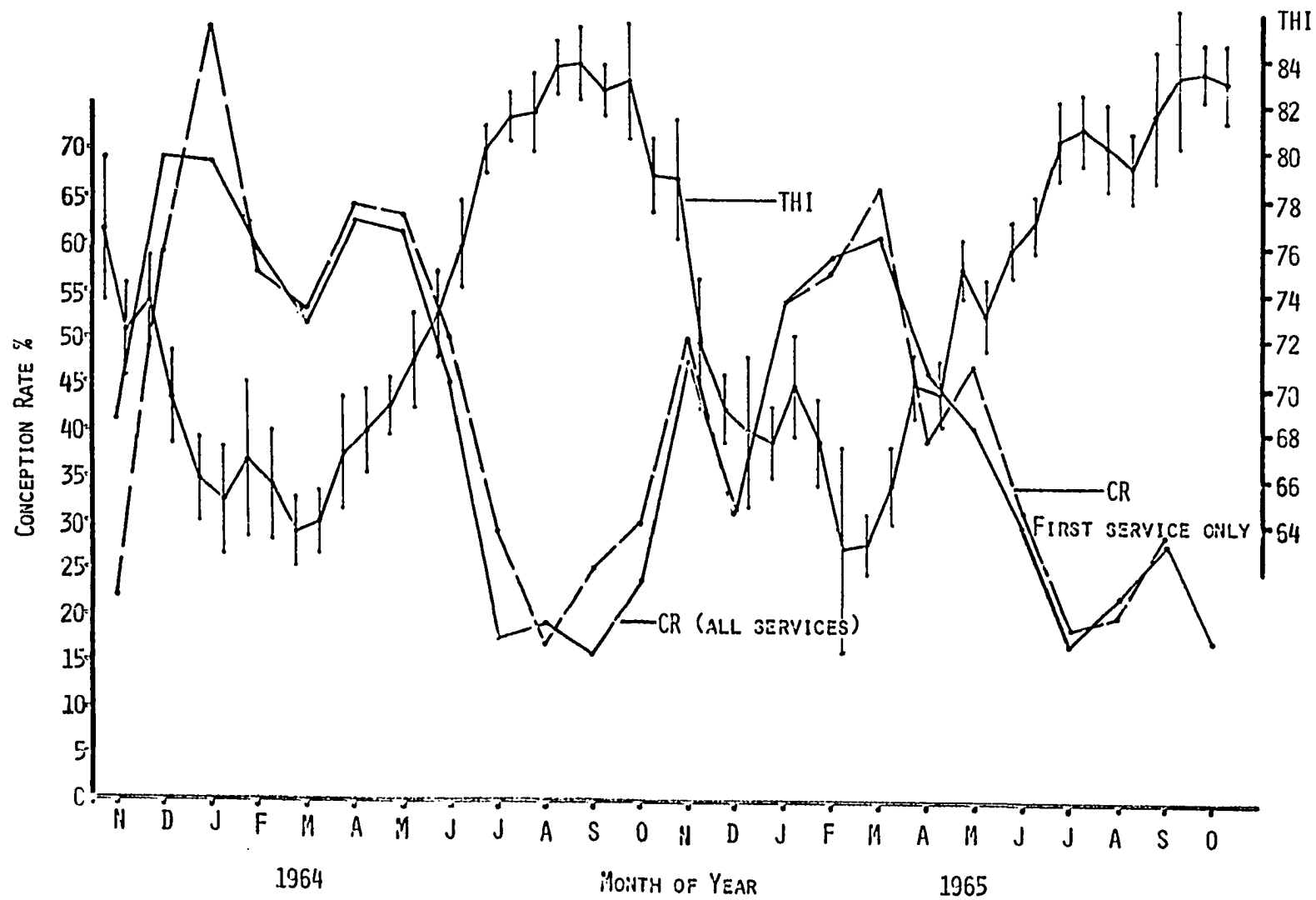


Figure 11. Average monthly conception rates and average THI ( $\pm$ SD) for 15-day periods for HW in 1967 and 1968

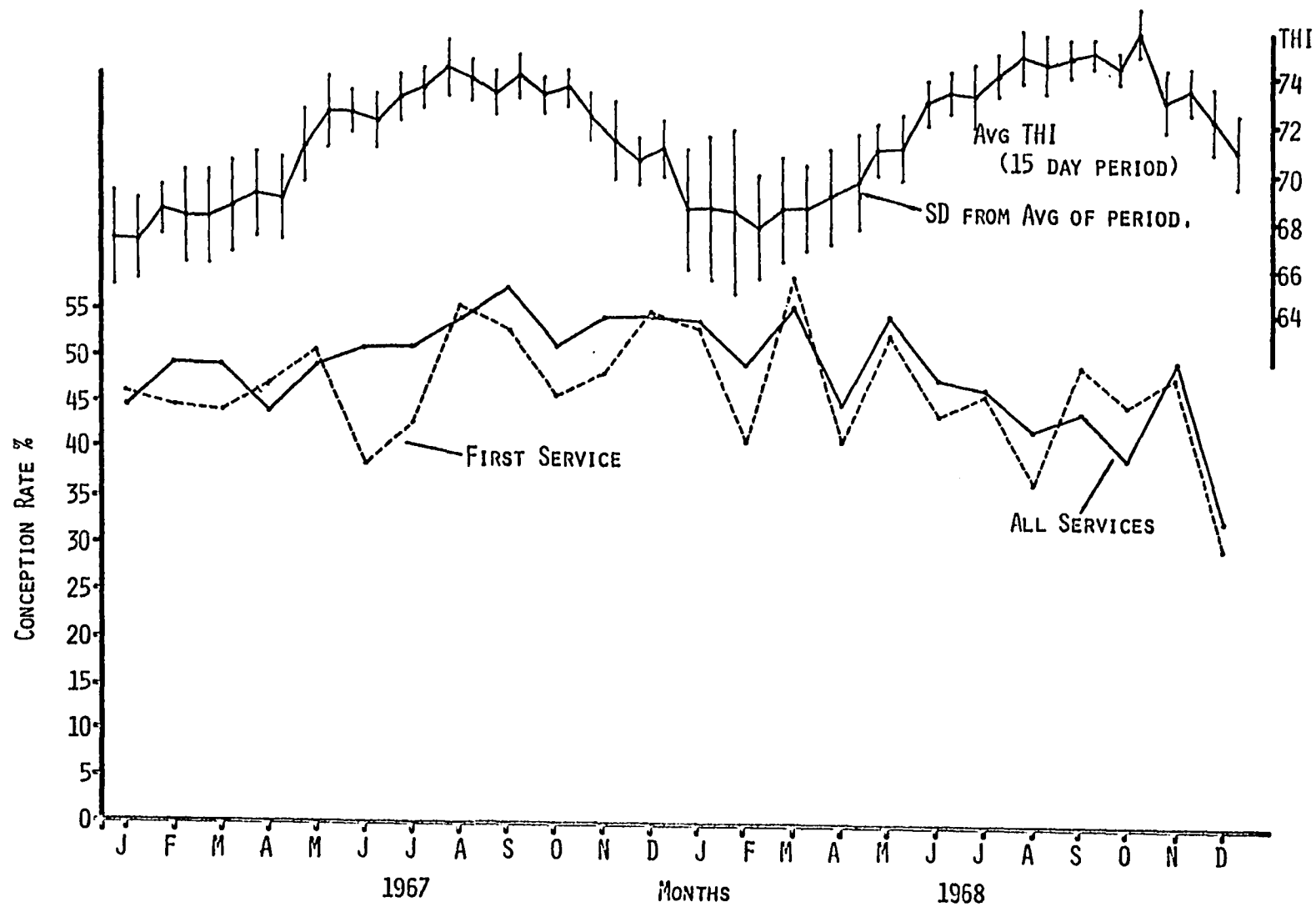


Figure 12. Conception rate and THI relationships in 1968 and 1969 for two dairies (HL) on the leeward side of Oahu, Hawaii. The solid line represents CR for all services and the dashed line represents CR for first service only for one dairy. Open circles represent all service CR on a neighboring dairy under different management

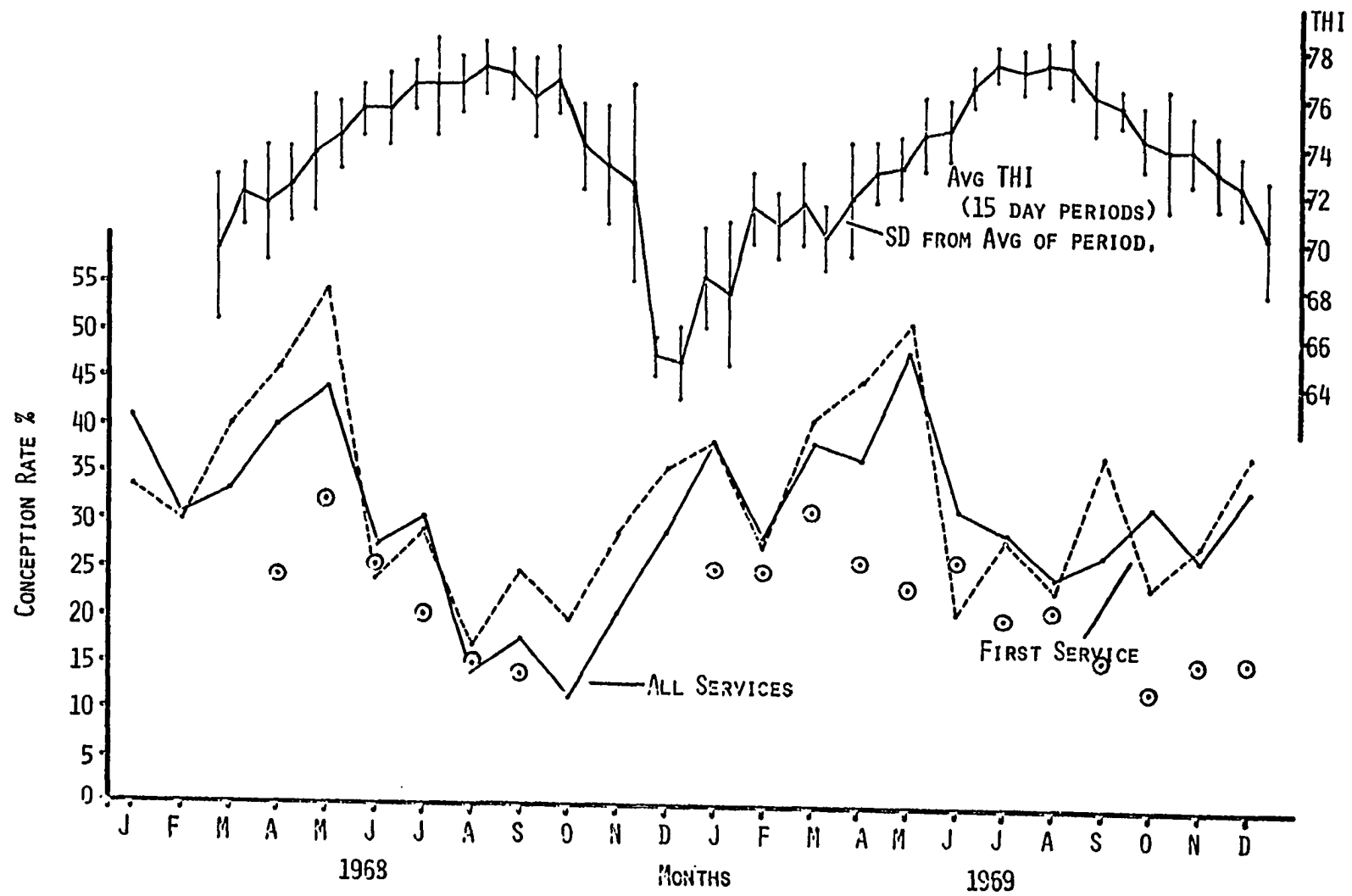


Figure 13. Conception rate versus the average THI of the day of breeding for 1966, 1967 and 1968  
at HW

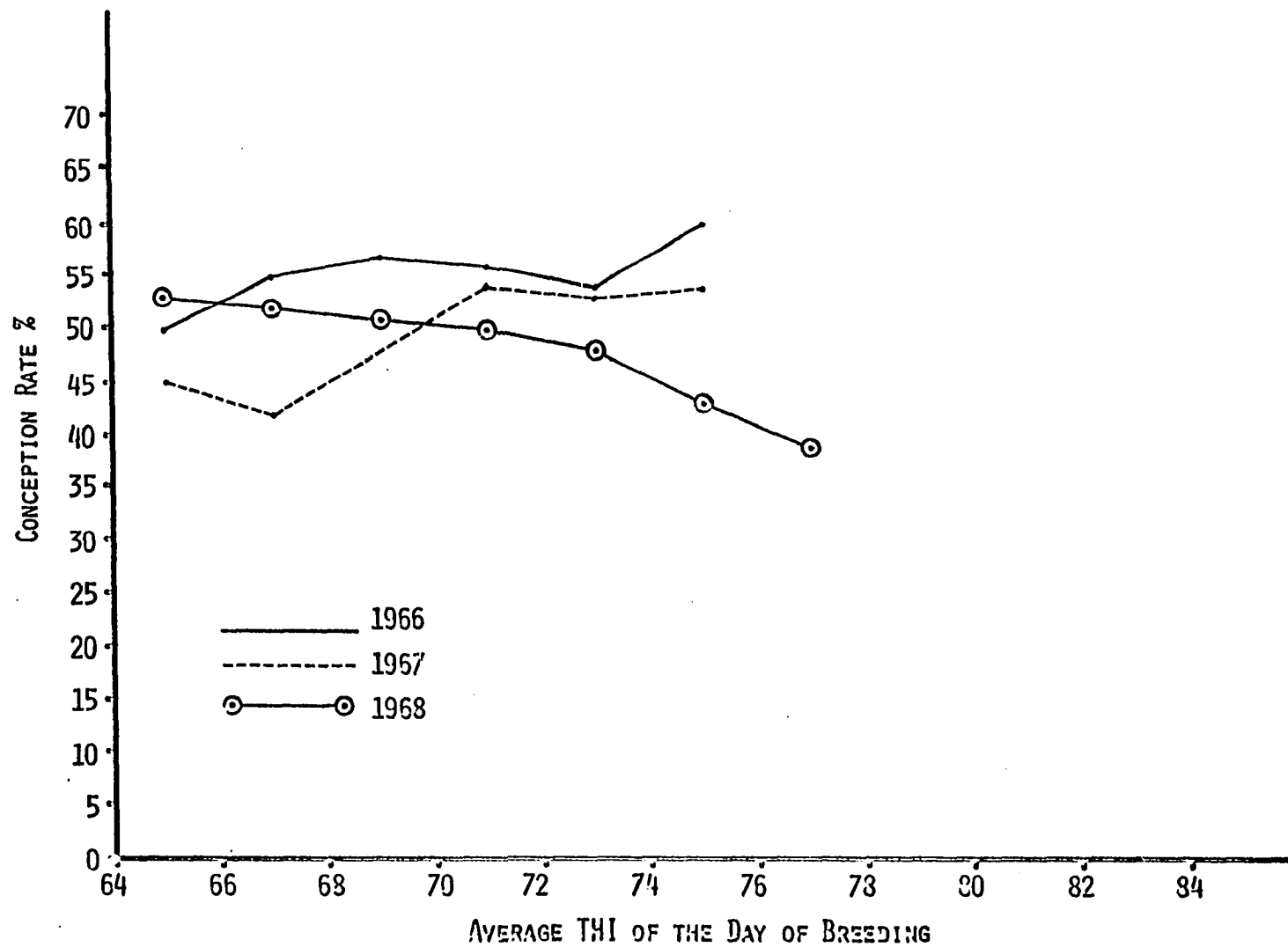




Figure 14. The response curve for CR versus THI of the second day prior to breeding (B-2) in 1966 has been split into two component seasons to demonstrate the relatively narrow range of THI during the warm season of that year. Sixty-two percent of the cows were inseminated at THI between 72 and 74 during the period from July 1 to the end of October. Numbers on the graph indicate the number of services at each point

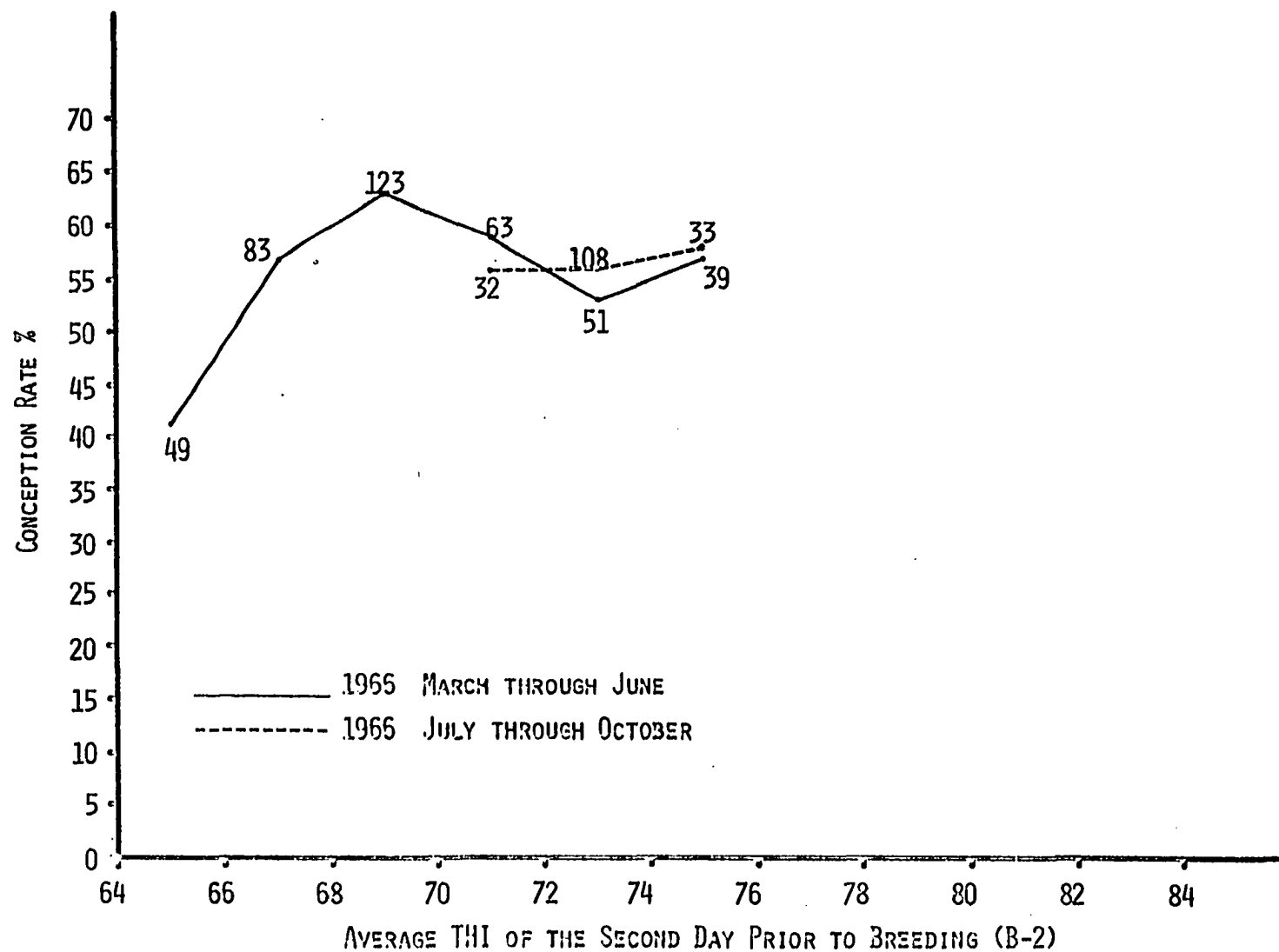


Figure 15. Conception rate versus the THI of the second day prior to the day of breeding (B-2) for MX. Data for full years (1963, 1964 and 1965) are included. Numbers at each point indicate the number of services at each point

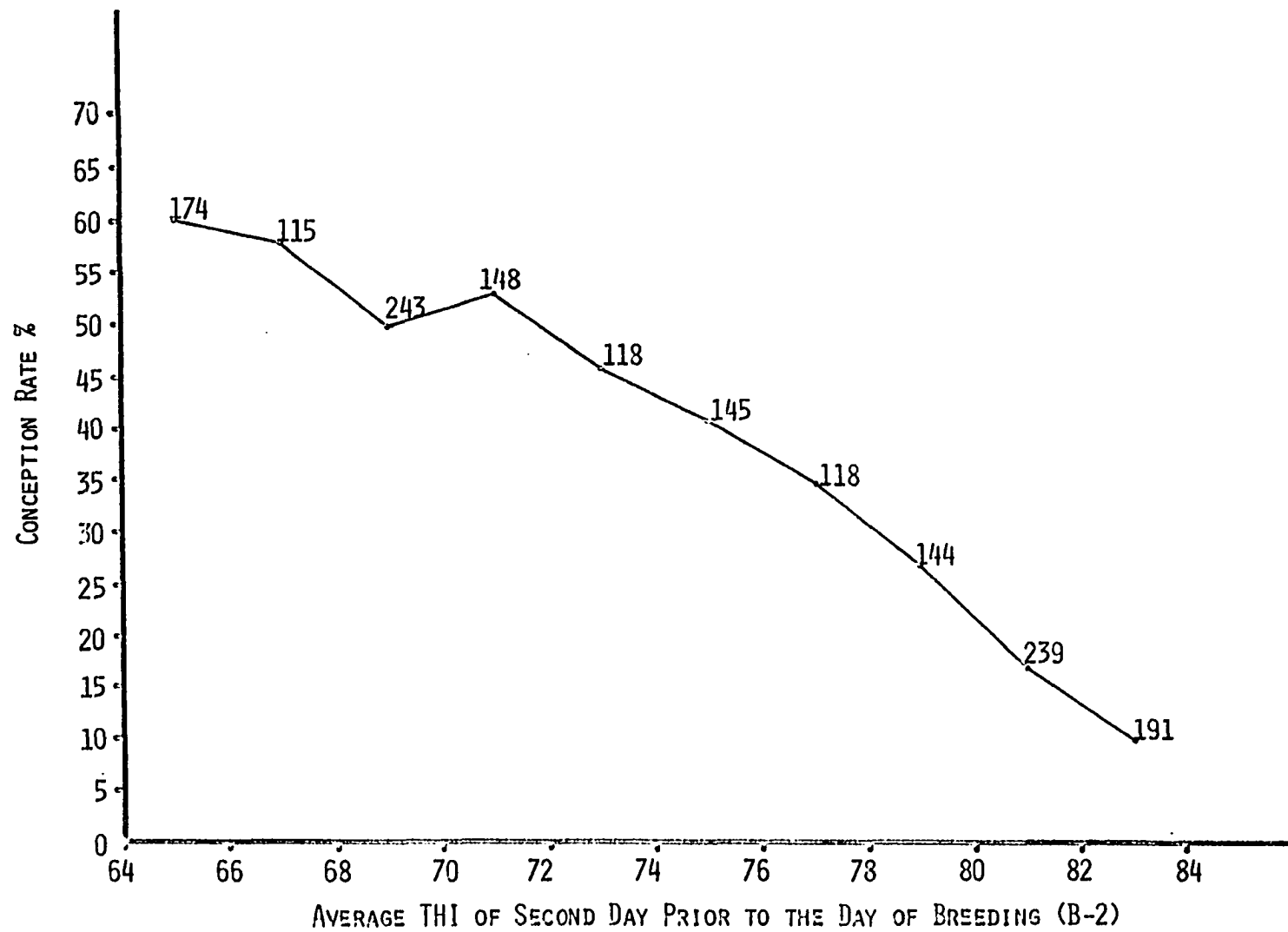


Figure 16. Conception rate versus the THI of the second day prior to the day of breeding (B-2) for MX. Data from Figure 15 have been split into two component seasons to demonstrate seasonal differences. Average daily THI tend to increase from May through September and decrease from September through January. Numbers indicate the number of services at each point

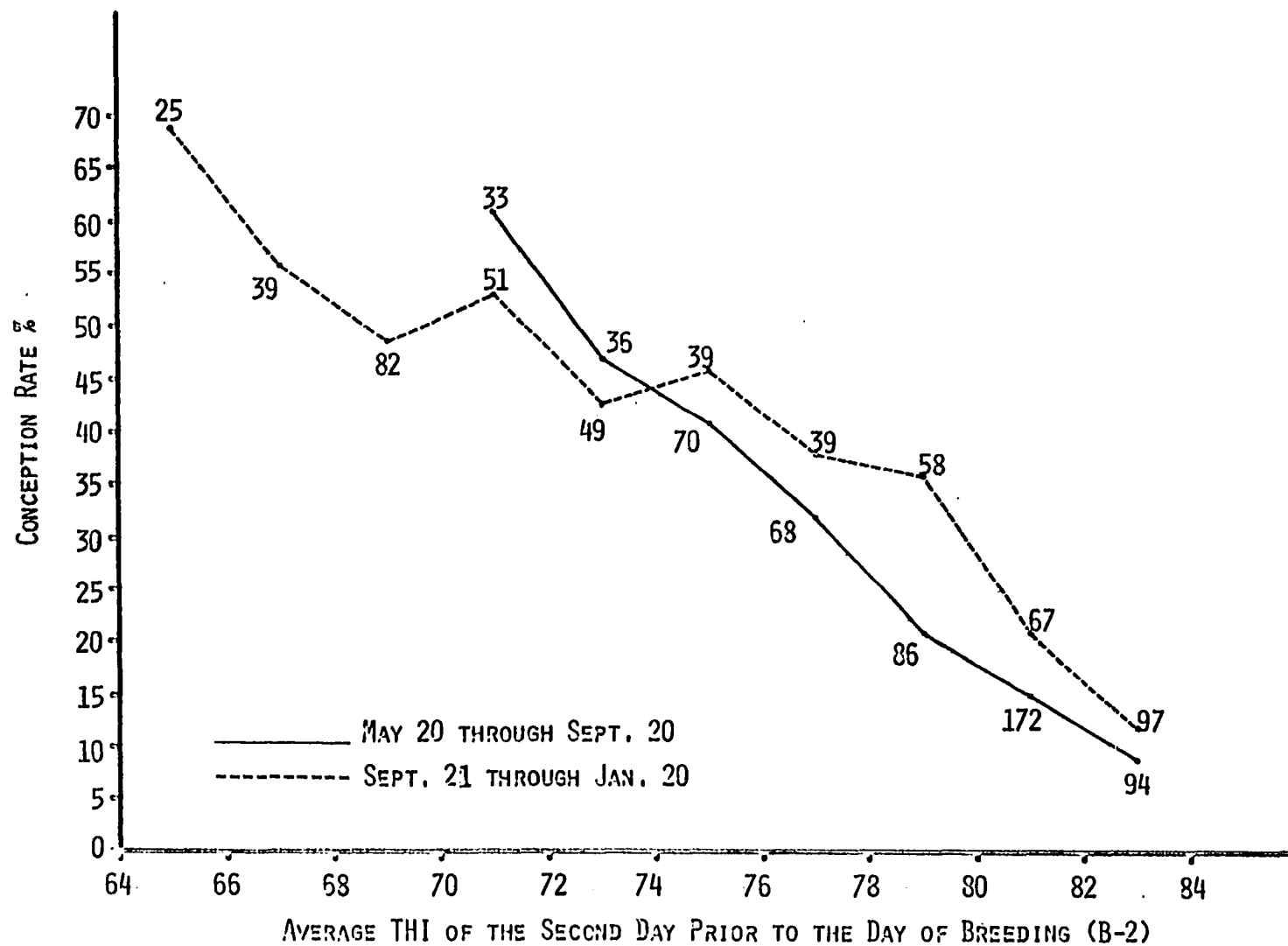


Figure 17. Conception rate versus the THI of the second day prior to the day of breeding (B-2) for HW. Data are for the full year of 1968. Numbers indicate the number of services at each point

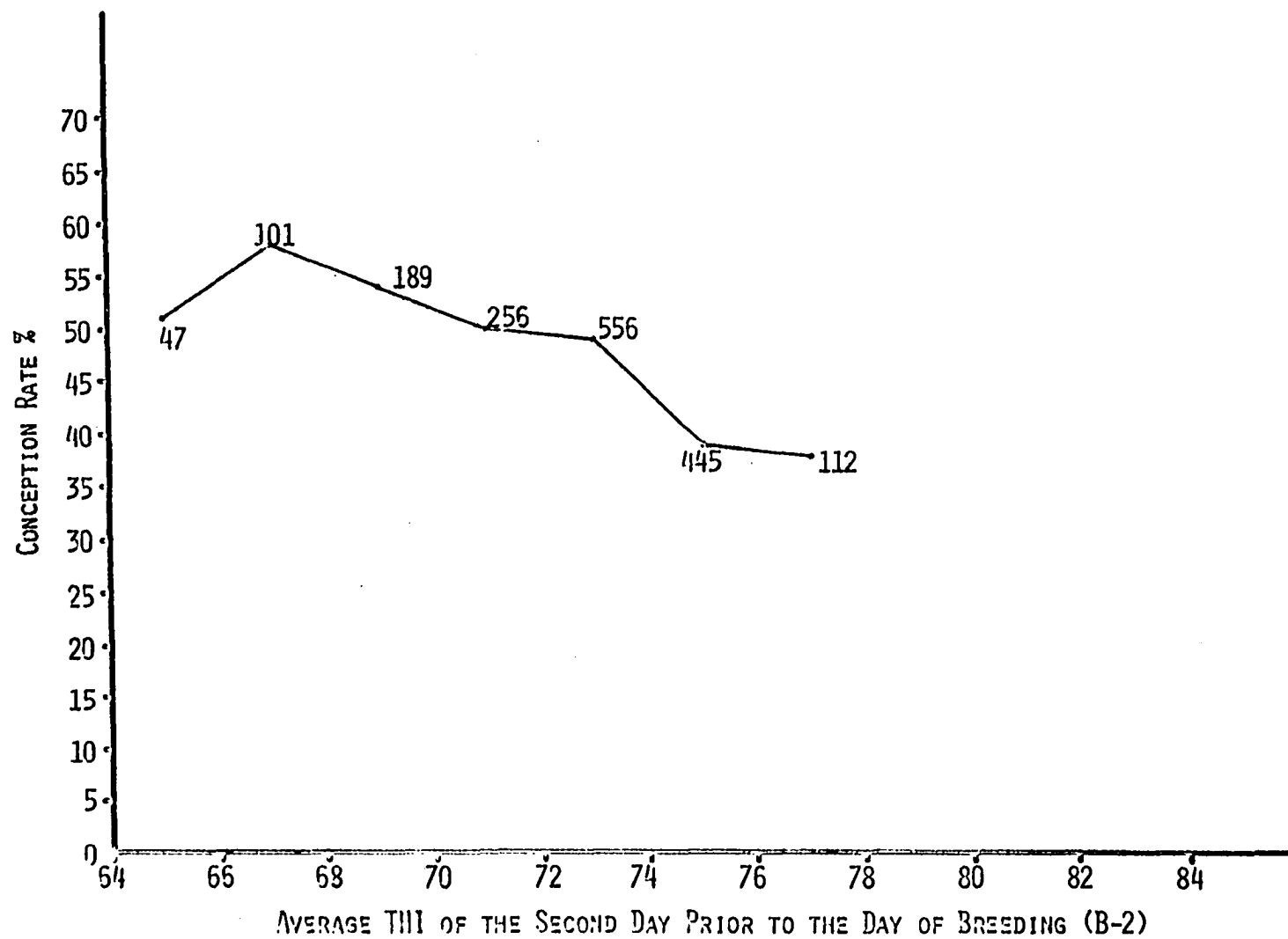




Figure 18. Conception rate versus the THI of the second day prior to the day of breeding (B-2) for HW. The data in Figure 12 have been split into two component seasons to demonstrate seasonal differences. Average daily THI tend to increase through both of these seasons. Numbers indicate the number of services at each point

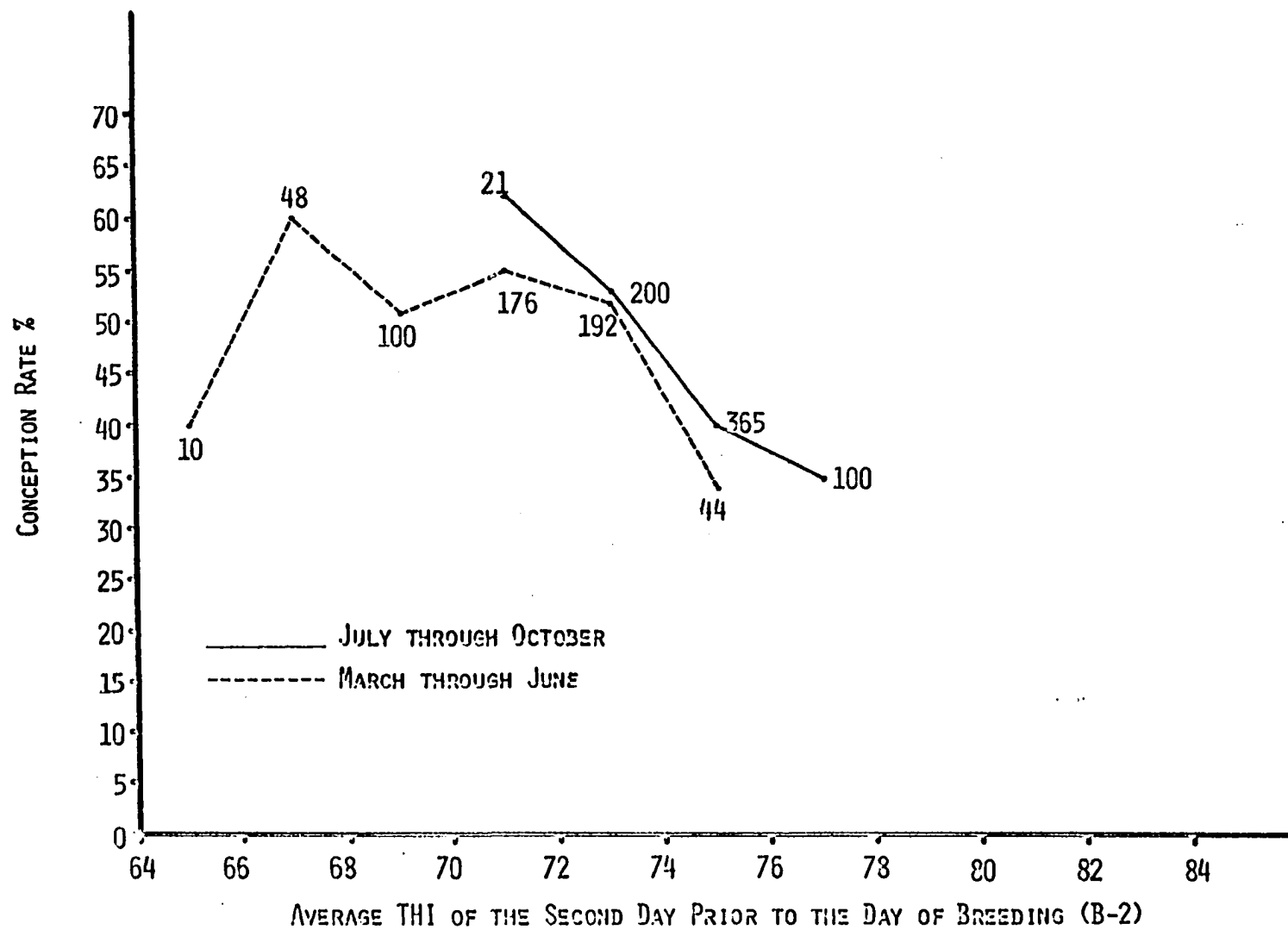


Figure 19. Conception rate versus the THI of the day prior to the day of breeding (B-1) for MX. Data have been split into those services in which B-2 was less than B-1 and those services in which B-2 was greater than B-1. Data for full years (1963, 1964 and 1965) have been included. Numbers designate the number of services represented at each point

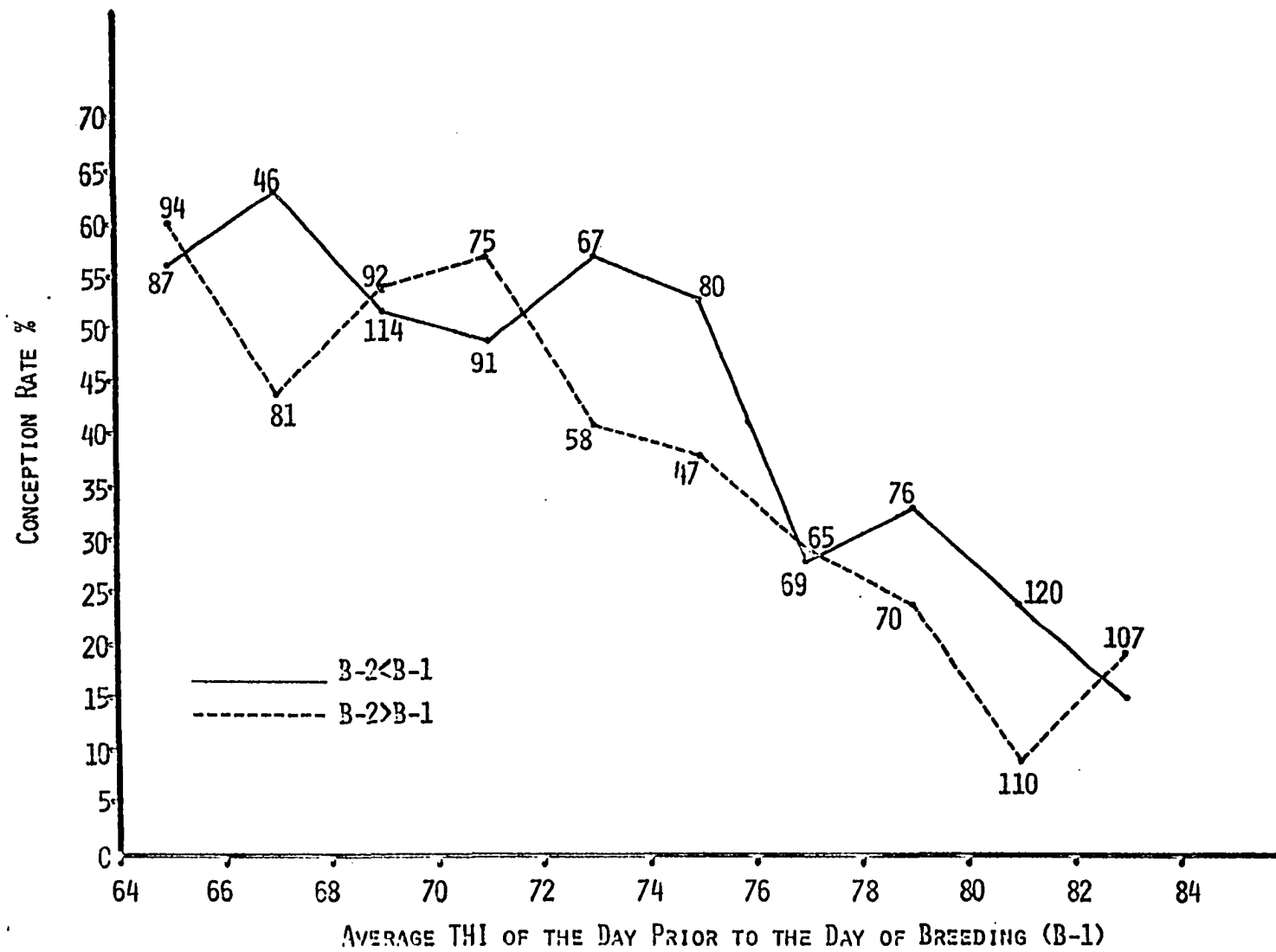


Figure 20. Conception rate versus the average THI of the day prior to the day of breeding (B-1) for HW. The data have been split into services in which B-2 was less than B-1 and services in which B-2 was greater than B-1. Based on all data for year 1968. Numbers indicate number of services at each point

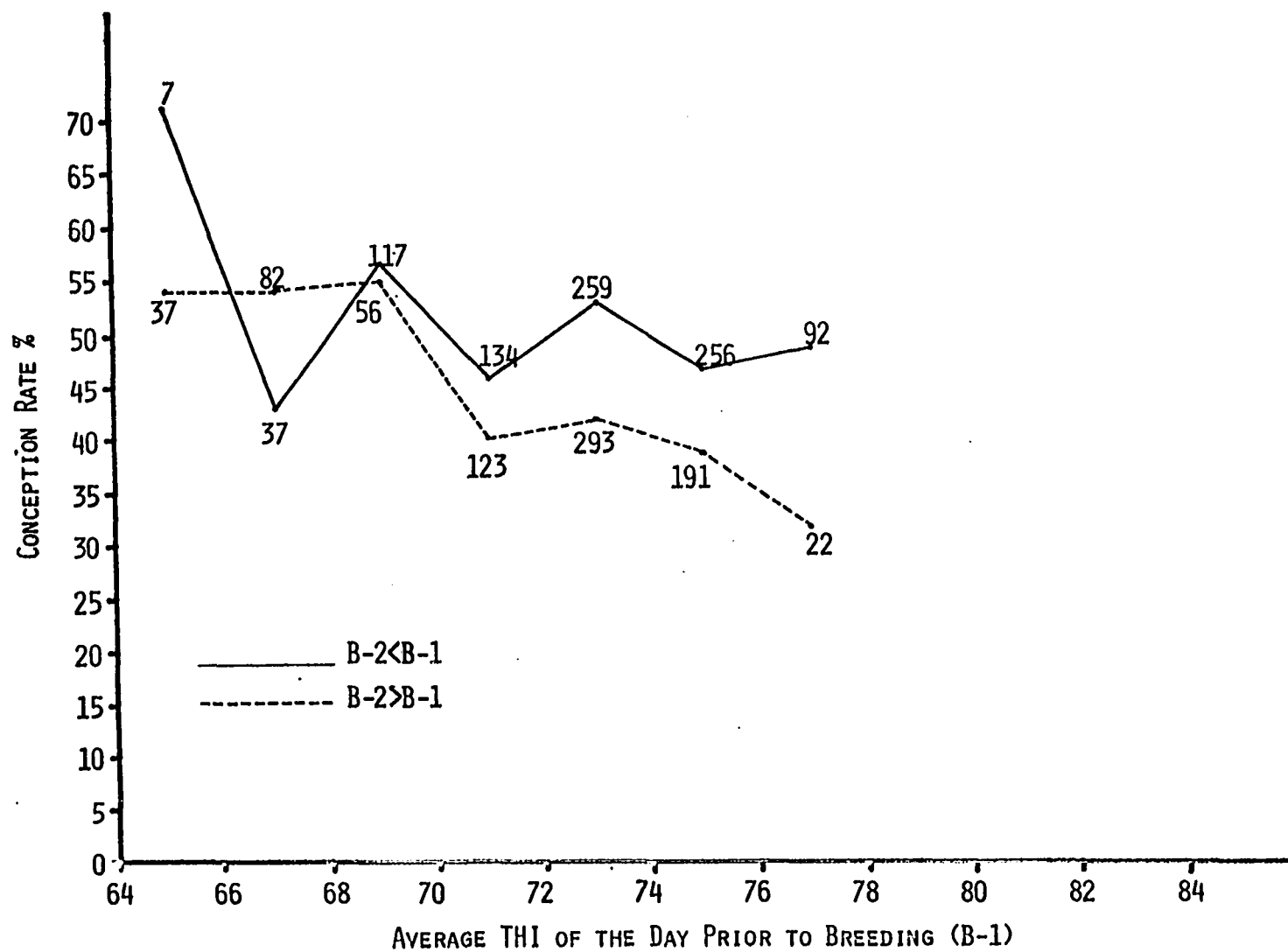


Figure 21. Conception rate versus the THI of the day prior to the day of breeding (B-1). The data have been split into those services in which B-2 was less than B-1 and those in which B-2 was greater than B-1

a. March through August, season of increasing THI at MX

c. March through August, season of increasing THI at HW

b. September through February, season of decreasing THI at MX

d. September through December, season of high and increasing THI at HW

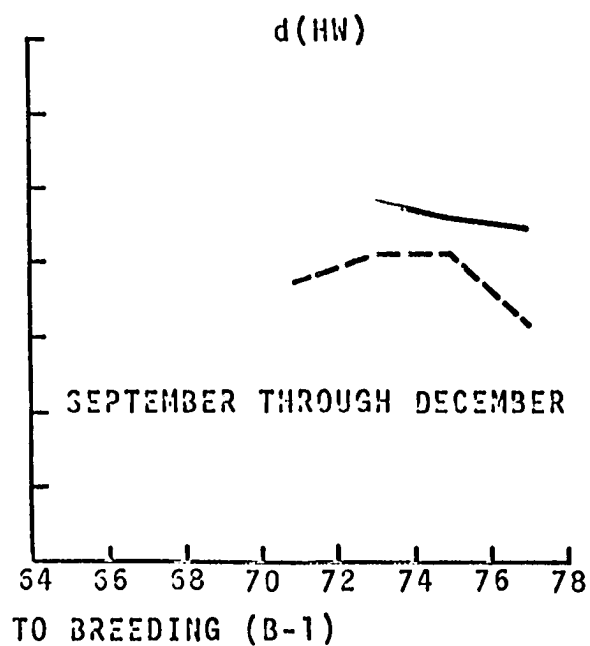
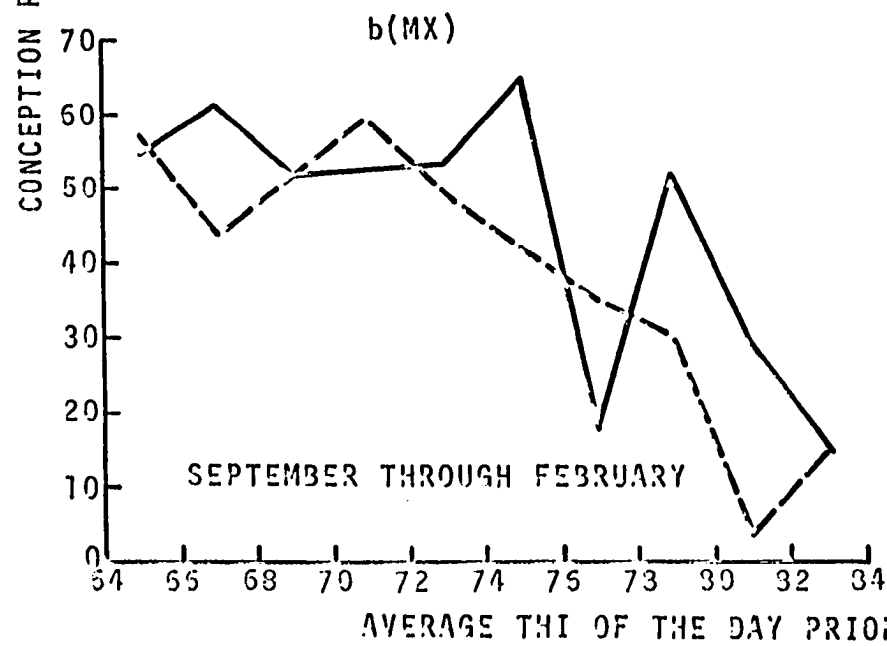
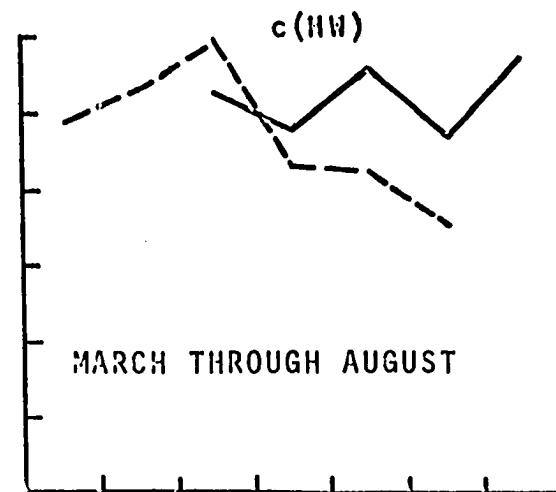
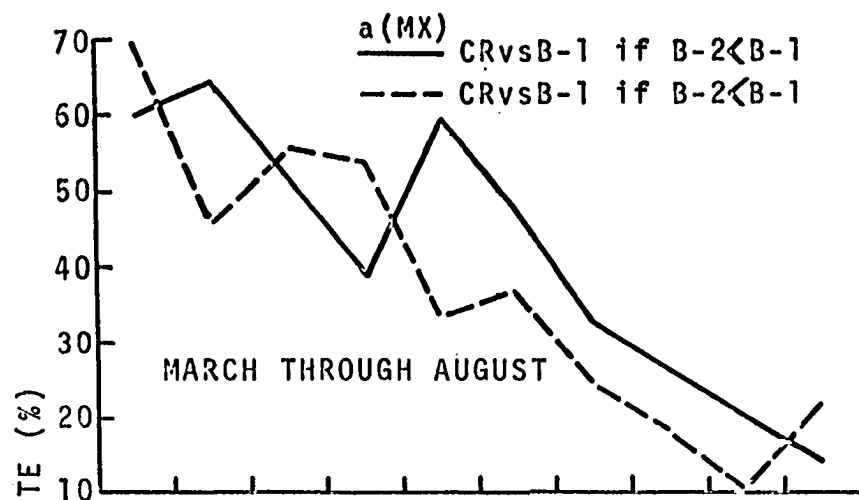




Figure 22. Conception rate versus the THI of the day of breeding (B) for MX. The data have been split into those services in which  $B-1$  was less than B and those services in which  $B-1$  was greater than B. Data for full years (1963, 1964 and 1965) have been used. Numbers represent the number of services at each point

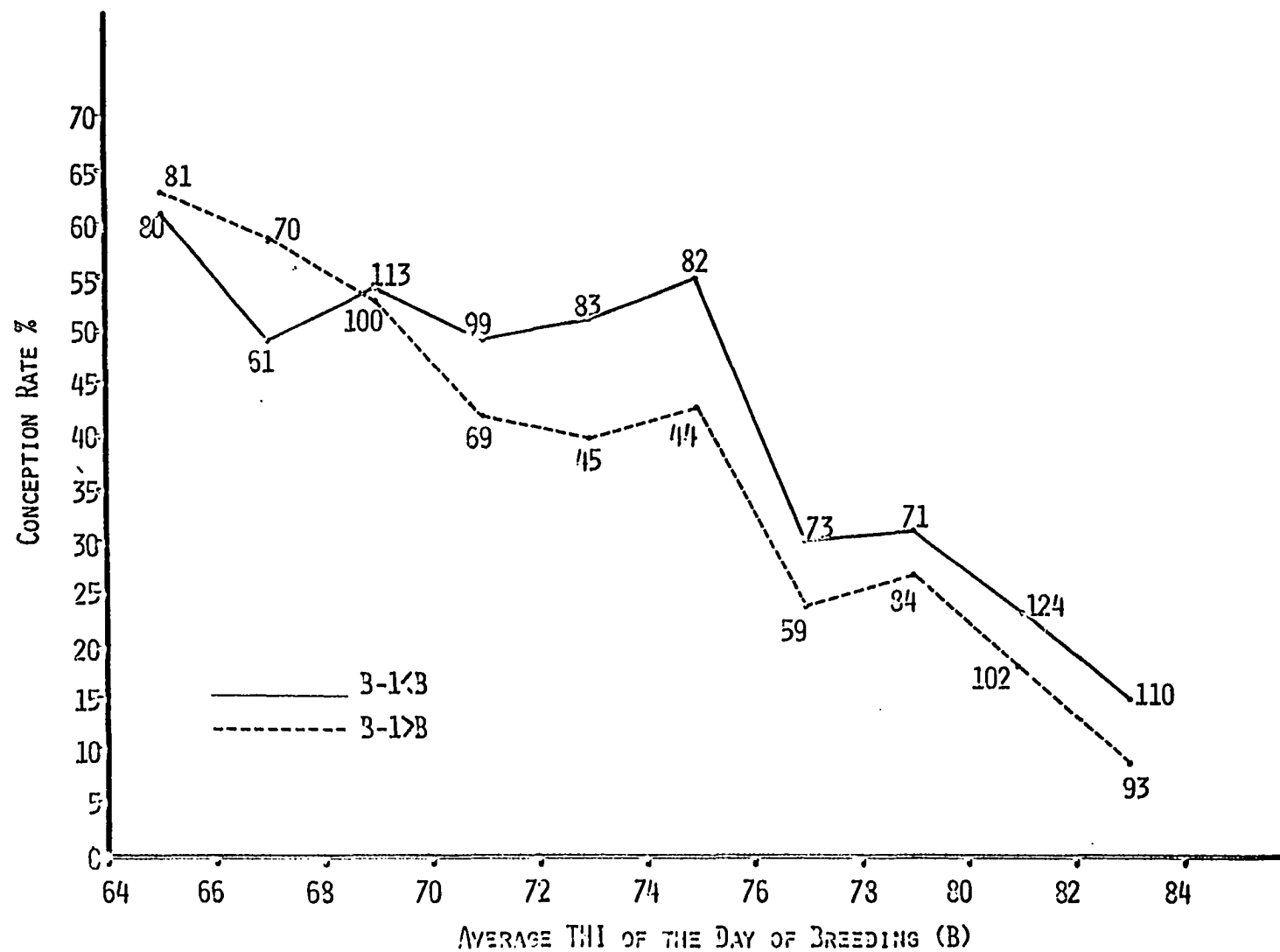


Figure 23. Conception rate versus the THI of the day of breeding (B) for MX. The additive effect which resulted if B-2 was greater than B-1 and B-1 was greater than B,  $B-2 > B-1 > B$ , is compared to the opposite situation in which  $B-2 < B-1 < B$ . Data for full years (1963, 1964 and 1965) have been used. Numbers represent the number of services at each point

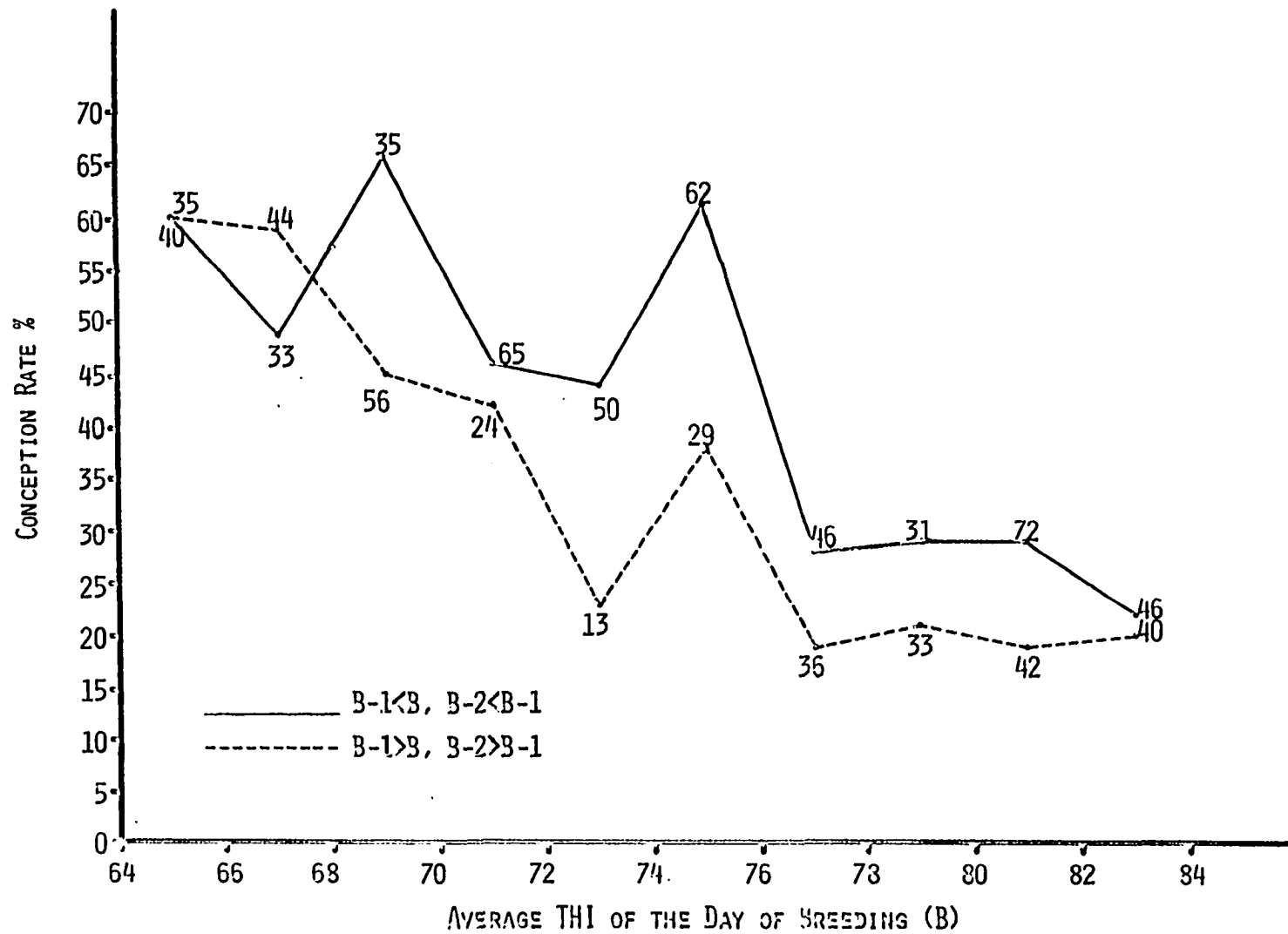


Figure 24. Conception rate versus the THI of the day of breeding (B). The data have been split into those services for which the average THI of the 2 days prior to the day of breeding,  $(B-2+B-1)/2$ , were greater than B and those services for which it was less than B. Data for full years (1963, 1964 and 1965) have been used

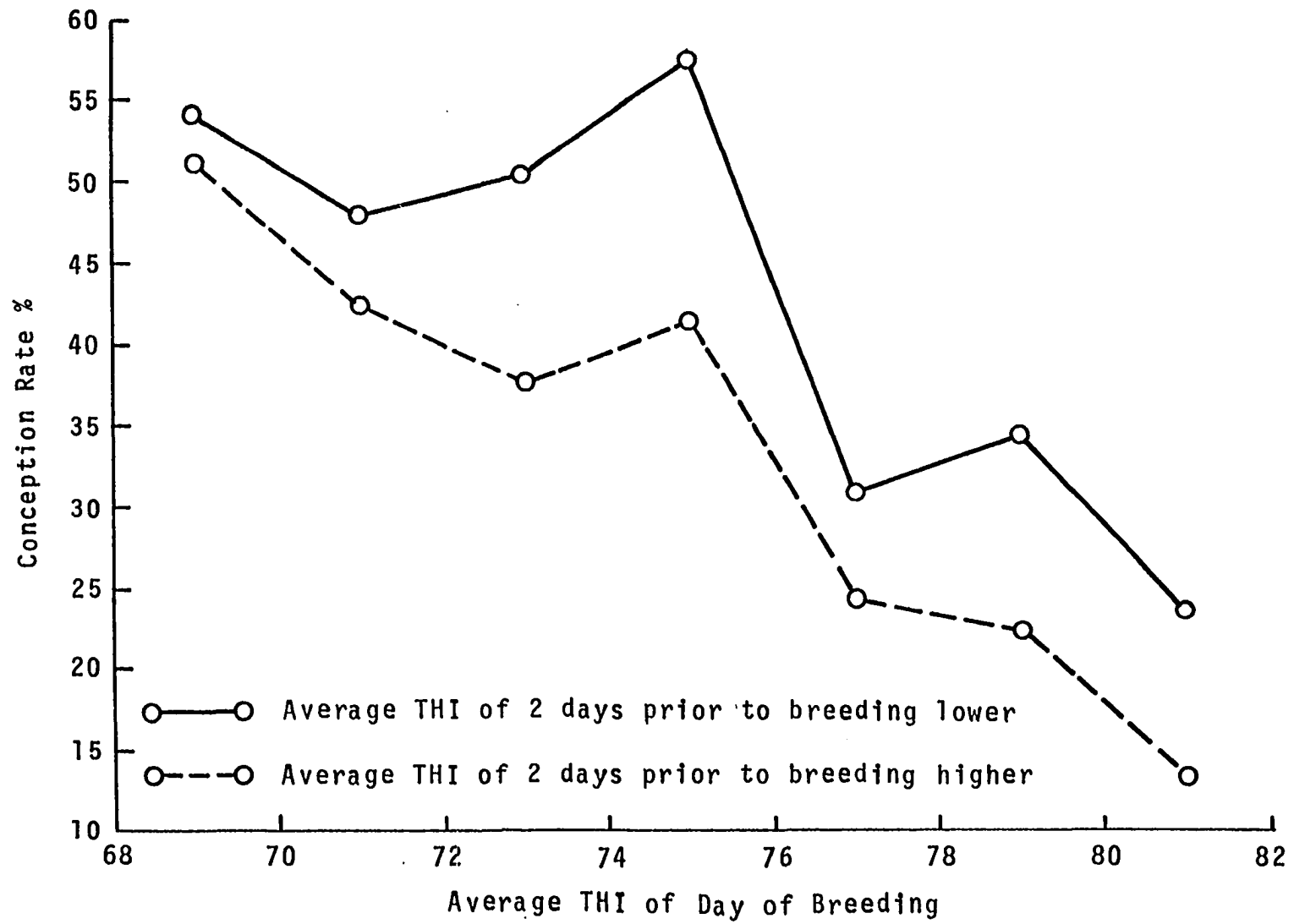


Figure 25. Conception rate versus the THI of the day following breeding (B+1) at HW. The data have been split into those services in which  $\bar{3}$  was less than  $\bar{3}+1$  and those services in which  $\bar{B}$  was greater than  $\bar{B}+1$ . Data for full year 1953 have been included. Numbers indicate the number of services at each point

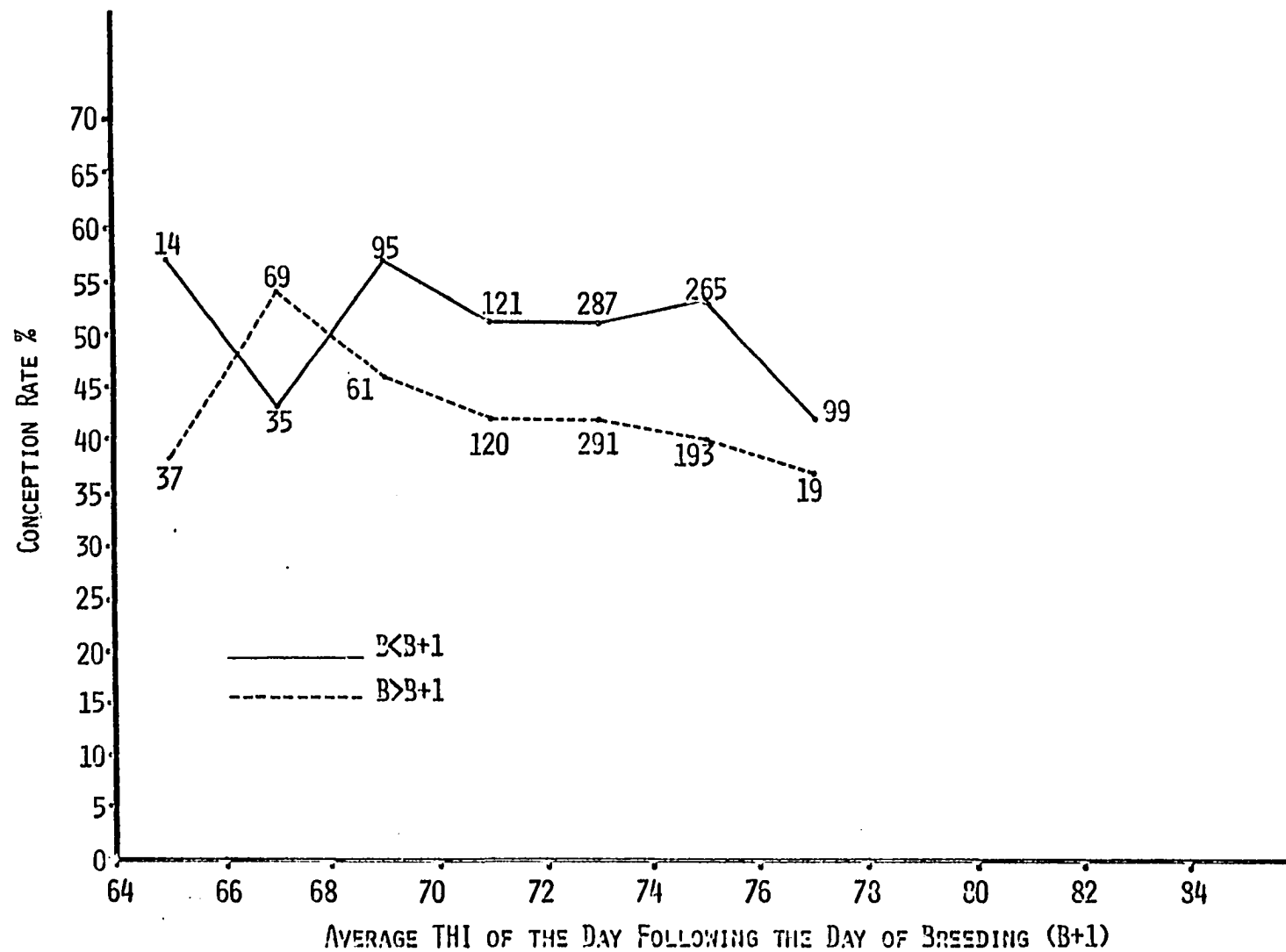




Figure 26. Conception rate versus the THI of the day following the day of breeding (B+1) at HW. The additive effect which resulted if B-2 was greater than B-1 and B was greater than B+1,  $B-2 > B-1$  and  $B > B+1$ , is compared with the opposite situation in which  $B-2 < B-1$  and  $B < B+1$ . Points for MX (Figure 19) are also included in the figure for comparison. Data for full year 1968 for HW are included. Numbers represent the number of services at each point

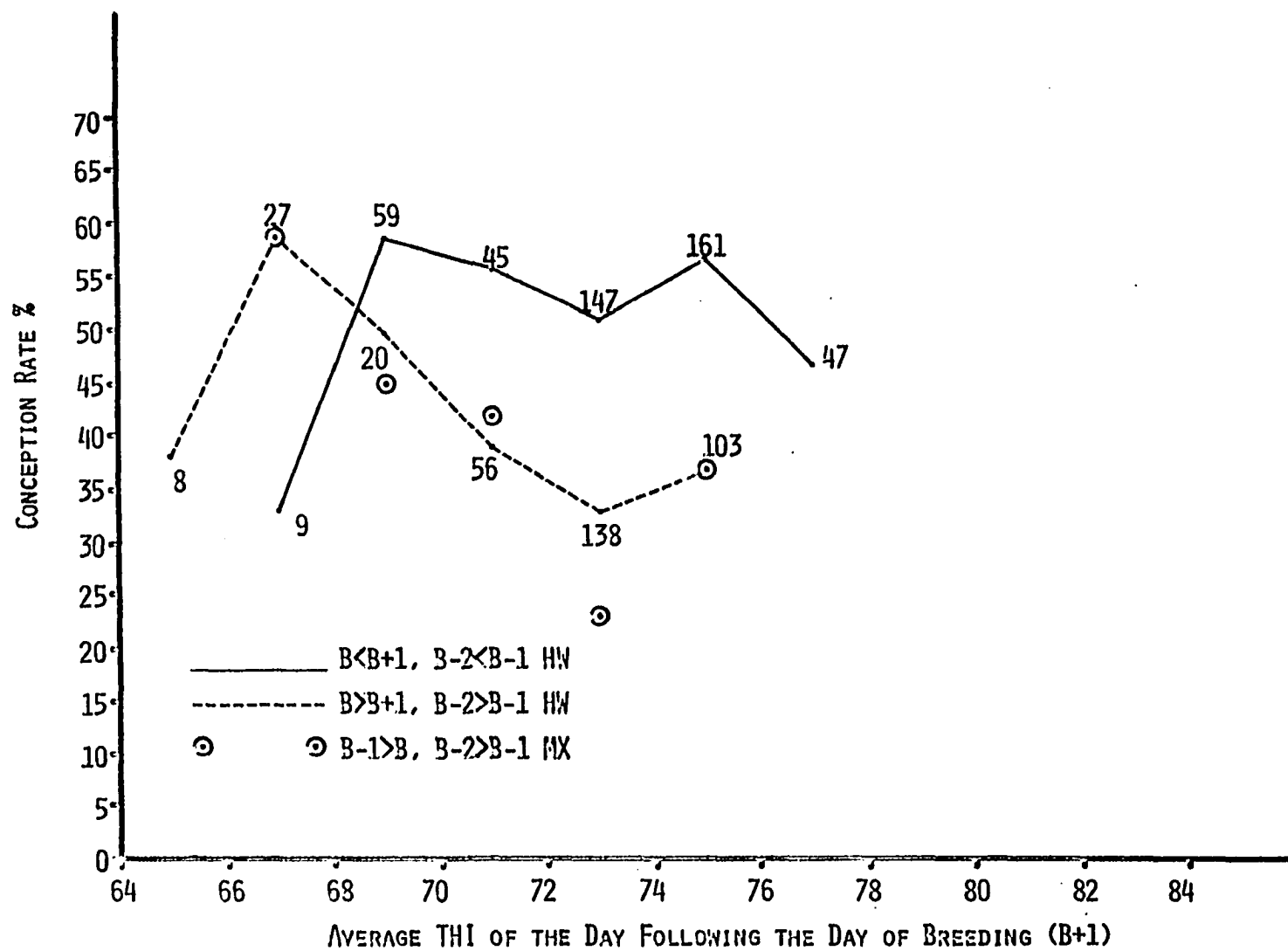


Figure 27. Conception rate versus the THI of the second (B-2), tenth (B-10) and fourteenth (B-14) day prior to breeding. Data are from Tables 21 and 22. Numbers represent the number of services at each point

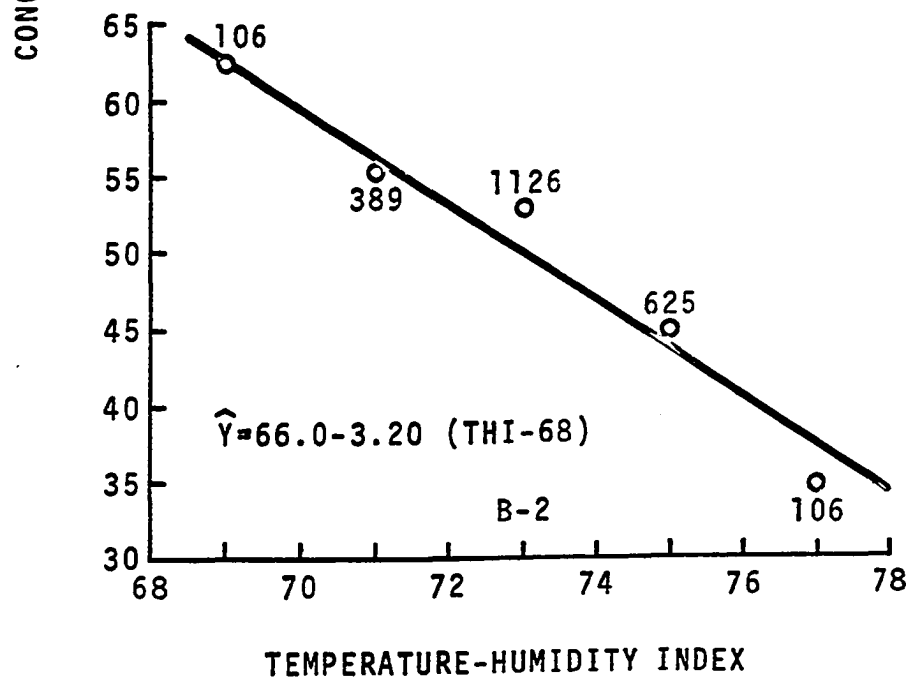
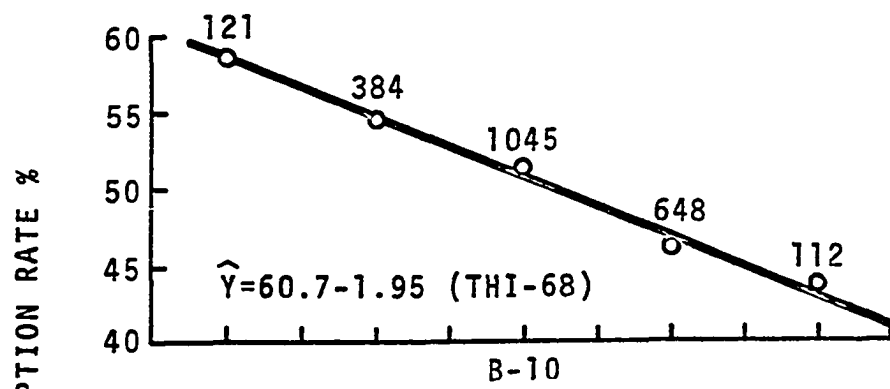
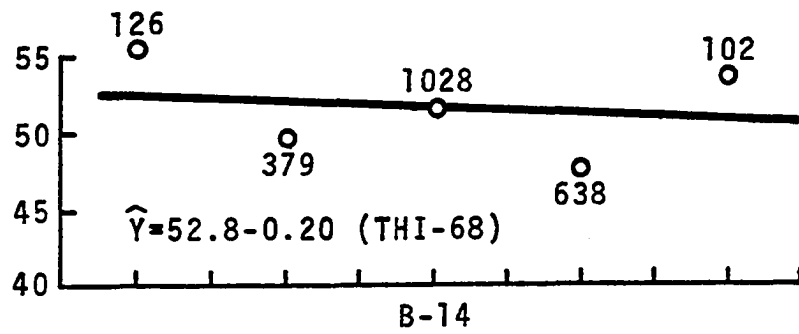


Figure 28. Slope of CR versus THI response curves for each of 16 days prior to breeding and 2 days following breeding for HW. Data are from Table 22

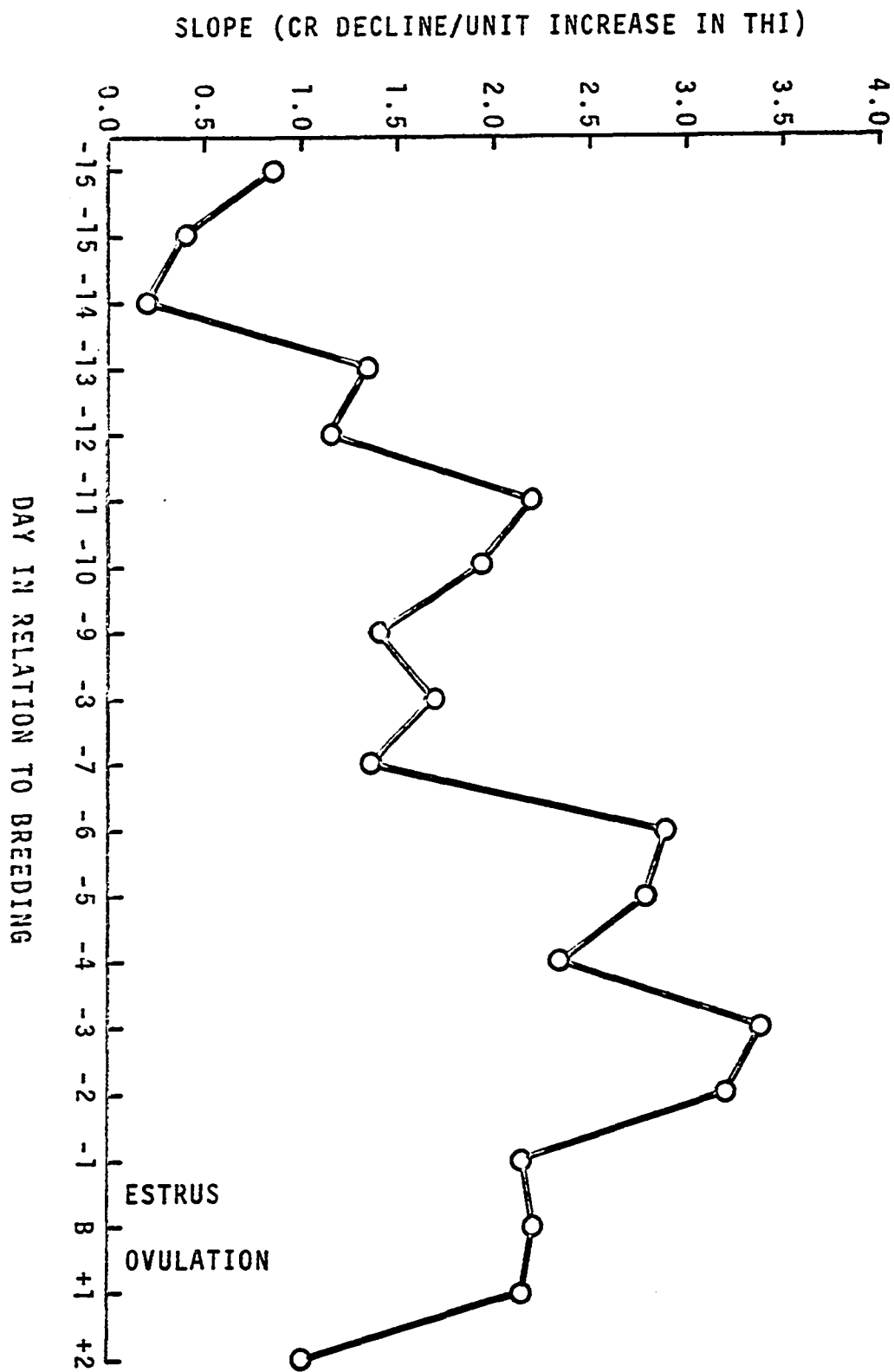
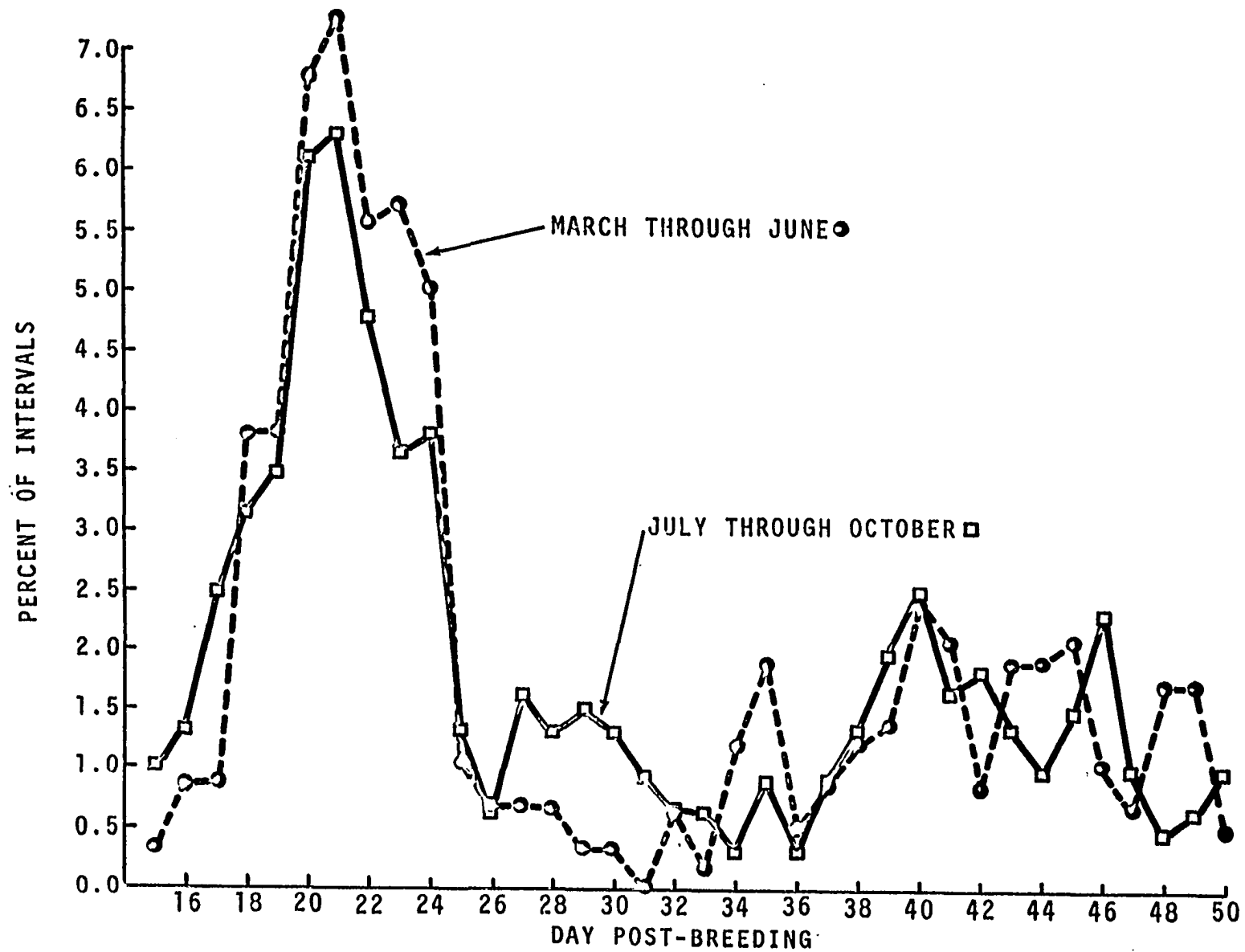


Figure 29. Percentage of the total number of intervals between services that were of a given length have been plotted to show the distribution of intervals of between 15 and 50 days duration. The intervals were between first and second service and second and third service at HW





## Discussion

Heat stress

Comparatively little information is available in the literature concerning the physiological response of cattle to day-to-day climate changes in the field. It seems logical to assume that the individual cow is in some degree of acclimation with the average THI of the preceding 2 weeks. Days with THI significantly above this average could be expected to cause some degree of acute stress and elicit exaggerated physiological responses. Three such responses (rectal temperature, milk production and TDN consumption) are shown in Appendix Table 114 (Ingraham, 1968). The average responses of 25 lactating cows to daily changes in THI over a period of 20 days in 1967 at MX are shown. The sharp average temperature increase (0.85 C) shown in this graph followed an average daily THI increase from 77 to 80. The days following varied over a range of only 3 THI units (78 to 81) yet significant changes occurred in all three variables. It was evident that the cows were having problems adjusting to these THI.

Data from both MX and HW appear to indicate that the majority of cows could acclimate reasonably well to THI under 76, although milk production and CR were depressed during the period of acclimation. However, when a degree of acclimation was achieved CR improved. Figures 13 and 14 (HW) show that relatively normal CR could be achieved at THI up to 76. The upper graph of Figure 24 (MX) indicates that the highest CR (57.5%) within the THI range of the graph (68 to 82) was achieved between THI 74 and 76. Figure 12 gives monthly THI-CR relationships for two dairies (HL) on the

leeward shore of Oahu, Hawaii in 1968 and 1969. Average THI for the warm season at this location was 76.4. Although this was only slightly higher than at HW (Figure 11), the warm season depression in CR was much more decisive, indicating once again that problems with CR depression may become more acute at THI above 76.

Rectal temperatures and CR Cows vary in their ability to maintain normal rectal temperatures at average daily THI above 76. This is demonstrated in Appendix Table 115 (Ingraham, 1968) which gives the cumulative distribution curves of rectal temperature at average daily THI 76.6, 78.5, and 80.3 for a group of 49 cows at MX. The percentage of the group with average rectal temperature (0230 and 1330 rectal temperatures averaged) over 39.7 C increased from 26% at THI 76.6 to 75% at THI 80.3. The linearity of the cumulative distribution curves ( $r = 0.98$ ,  $P < .001$ ) of this group of lactating Holsteins stimulates speculation that CR is a function of the percentage of the cow population with body temperature above some critical figure. However, a look at Table 22 indicates that there were at least 13 days with a significant relationship between CR and THI at HW. Conception rates were not equally responsive to the THI of each of these days. This raises the possibility of a different critical body temperature for any one of 12 or more critical days.

Several investigators (Long et al., 1969 and Gwazdauskas et al., 1972a) have reported a significant relationship between a rectal temperature taken on the day of breeding and CR. At rectal temperatures above 39.7 C, CR were depressed significantly. Abdominal temperatures would very likely be 40 C or higher at rectal temperatures above 39.7 C. Since Alliston et al.

(1965) demonstrated that rabbit ova were damaged at 40 C, there is good support for the thesis that high body temperature might damage the cow ova. However, in the light of the present study, perhaps too much emphasis was placed on the significance of the time that the temperature was taken. It would also be of interest to know the level of rectal temperature that preceded breeding. Until more information is available, it would probably be prudent to consider single rectal temperatures taken at breeding as an index of the ability of the animal to adjust to the climate rather than something that is necessarily causing damage at that time.

Acclimation and CR      The effect of the relative acclimation of the cow to the THI at breeding on CR is given in Figures 16 (MX) and 18 (HW). They indicate that CR improved with acclimation. For example, from May through September at MX the average CR at THI 76 was 21% while from September 20 to January 20 the average CR at THI 76 was 36%. The increase in CR was probably related to the THI levels to which the animals were acclimated at breeding. Since THI were decreasing from September to January, many of the cows inseminated at THI 76 were probably acclimated to THI as high or higher than this. This implies that the average THI to which the cow is acclimated, as well as the actual THI involved with breeding, must be considered when an estimation of CR response to climate is made.

#### Heat stress and fertility

The majority of investigations concerning heat stress and fertility have been limited to the post-ovulation period. Consequently, theories on the manner in which conception rates are reduced by heat stress have, for

the most part, concerned the effect of the environment of the oviduct or uterus on the developing ova. Two schools of thought persist. One emphasizes the damaging effects of thermal stress directly on the gametes and the other considers the manner in which thermal stress alters the hormonal environment that is responsible for the normal development of the embryo. There is evidence supporting both theories but a model explaining the latter effect, involving hormones in the post-ovulatory period, has avoided elucidation. On the other hand, a logical model for the manner in which alteration of hormonal activity might depress sexual activity is available and will be discussed later.

Direct effect of thermal stress The results of this study at MX and HW add a new dimension to the speculation for they indicate that heat stress in the pre-ovulatory period may be as important, if not more so, than the period after ovulation. This change in emphasis raises new questions about the effects of local thermal stress on follicle development in the ovary. Specifically, would estrogen and/or progesterone production be altered if the thermal environment of the ovary were increased above normal? Would the fluid environment of the follicle be altered? If it were, could this result in an abnormal maturation of the ovum?

Although answers to these questions are not available there is precedent indicating that locally applied heat can cause damage to gametes, namely in the male testes. Mazzari (1968) depressed semen quality in boars by heating (40.5 C) their scrotums for 3 hours. Moule and Waites (1963) reported that depression of semen quality in heat stressed (40.5 C) rams was related to a rise in the temperature of the subcutaneous tissues

of the scrotum rather than rectal temperature. When rams were given the same heat stressing treatment while their scrotums were cooled in a water jacket there was no depression in semen quality.

Since testicles atrophy if transferred to the body cavity, a thermal environment comparable to that of the ovary, a direct comparison of the response of the two gonads to thermal treatment may not be appropriate. However, the limits in thermal environment for a normally functioning ovary may in fact be rather narrow. Nalbandov (1964) suggests that a rat ovary transplanted on the ear or tail of a castrated rat may produce testosterone, indicating that temperature may influence the enzyme systems for steroid production in the gonad. Information on steroid production in the ovary at body temperatures above normal is apparently not available. Circumstantial evidence that temperatures above optimum may depress ovarian function lies in the fact that the left ovary of the cow and sheep is less active than the right ovary. Brody et al (1955a) reported that temperatures in the upper rumen, which lies in close proximity to the left ovary, were 1.23 C above rectal temperature. It seems plausible that the local heating effect of the rumen could suppress the enzyme systems necessary for hormone production in the ovary. Further support for this thesis is given by the fact that the right ovary has been reported (Hafez, 1968) to be less active than the left ovary in the mare and the sow. The site of fermentation for the horse which would be comparable to the rumen is the cecum. For the sow it is the large intestine. Both lie in close proximity to the right ovary.

Rajakoski (1960) compared follicular activity in the right and left ovary of the cow and reported that the greater functional activity of the right ovary involved only the maturation and ovulation of follicles equal to or greater than 5 mm. Smaller follicles occurred in both ovaries to the same extent. This would indicate that there was less stimulus for follicles to develop beyond this size in the left ovary. Unless the blood supply to the left ovary is suppressed in some manner, both ovaries should be receiving the same stimulus from pituitary gonadotropins. One possible explanation would be that thermal stress by heat from the rumen suppresses the ability of the left ovary to respond to the stimulus of the gonadotropins. Estrogen production and consequently follicle growth might be depressed as a result. An experiment that would determine the validity of this thesis is within the scope of present techniques and could add substantially to the understanding of ovarian function.

Additional support to the thesis that increased body temperatures may suppress steroid production comes from the suggestion by Marple et al. (1972b) that ACTH production may not be depressed in heat stress. If this is true, reduction of plasma corticoids in heat stress may not be the result of a lack of stimulus from the pituitary, but rather a reduced ability of the adrenal cortex to respond to ACTH. As has been suggested for the ovary, local thermal stress may depress the enzyme systems for steroid production.

Follicular activity during the estrous cycle      The thesis that local heat stress may suppress ovarian activity fits the present study, especially if the implications of Figure 28 can be interpreted as indicating a changing sensitivity of the developing ova to thermal stress. This

graph appears to follow the waves in follicular growth that Rajakoski (1960) proposed occurred within the estrous cycle. He theorized that follicles equal to or greater than 5 mm in diameter were exposed to two growth waves, the first reaching a peak on days 3 and 4 of the cycle. Following upon this growth phase he suggested that one large normal follicle persisted on the ovaries up to the eleventh day of a 21 day cycle. In Figure 28 this would be approximately the eleventh day prior to breeding and would correspond with the first peak on the graph. He suggested that all other growing follicles underwent atresia between the fourth and seventh day of the cycle (B-16 to B-14 of Figure 28). He further proposed that a second wave of follicle growth to exceed 5 mm commenced on the twelfth day of the cycle and once again one large follicle would emerge which he considered to be the one that would ovulate at the subsequent estrus. Growth of this follicle would correspond with the last peak on Figure 28.

Marion and Gier (1971) reported finding no waves of follicular growth as suggested by Rajakoski (1960) and contended that follicular growth was continuous and independent of the phase of the cycle. However, their graph of follicular growth and atresia during the cycle is in reasonably good agreement with the one given by Rajakoski. The difference appears to be one of interpretation of the data.

Hansel et al. (1973) have supplied further support to the contention that there are cycles of follicle growth during the estrous cycle. They reported three "peaks" of plasma estrogens during the estrous cycles of cows and sheep. They also commented that these elevated levels of plasma estrogens corresponded, in a rough way, with periods of accelerated

follicle growth that have been described by Rajakoski (1960) and Marion and Gier (1971). The first estrogen peak occurred during the first few days of the cycle before plasma progesterone began to rise. The second estrogen peak occurred 7 to 10 days prior to plasma progesterone decline and may be related to the increased sensitivity of CR to THI that occurs between the ninth and twelfth day prior to breeding in Figure 28. The final estrogen peak was considered to be involved with the growth of the ovulatory follicle and responsible for "triggering" the release of ovulatory amounts of LH from the pituitary. In the cow it began on the fifth day prior to estrus. This would correspond to the sixth day prior to breeding in Figure 28 and would correlate with the beginning of the second peak on that graph. Plasma LH also began to increase at this time. On the second and third days prior to breeding (the days indicated most sensitive to increased THI in Figure 28) progesterone is declining rapidly while estrogen is increasing rapidly. On the day prior to or early on the day of breeding estrogen levels were high and LH levels were increasing sharply.

In summary, the preceding reports and Figure 28 might be interpreted as indicating the following. (1) There are cycles in follicle growth within the estrous cycle. (2) These growth cycles are coincidental with increased plasma estrogen. (3) At the peaks of these cycles the factors in the ovary which control the normal development of the ova are most sensitive to thermal suppression. More specifically, thermal stress might decrease the ability of the ovarian cells to respond to the stimulus of the gonadotropins. This would not preclude a direct effect of thermal stress



on the ovum itself nor would it preclude the thesis that the level of gonadotropin might also be depressed.

Short term effect of heat stress Each of the above theories specifies that suppression of the ovum or its supporting system occurs only during the time of heat stress. That is, the reproductive efficiency of the cow is not altered over a period of days beyond the current estrous cycle following exposure to heat stress in the ranges found at HW and MX. If such were the case, and an extended period was necessary for recovery, the THI of an individual day would contribute comparatively little to CR response. Depressed CR could be considered a seasonal phenomenon that might be related to the weather of that season. For example, since a THI above 76 only occurs during the warm season, a means of determining an effect of THI 76 on CR that is independent of that season was necessary if the effect of THI alone was to be established.

One approach was to determine the relative sensitivity of CR to the THI of several days before and following breeding. It was theorized that if the slopes of the CR versus THI response curves of some of these days were significantly greater than others it would reflect an independent effect of the THI of those days on CR. The slope of CR versus B-2 as compared with CR versus B+2 (Figure 28) is an example.

A second approach was to evaluate the effect of the THI of one day on the CR versus THI response curve of the following day. A series of such evaluations are given in Figures 19 through 26. They indicate that the THI of individual days can have a decisive effect on CR.

Determination of a critical period

The CR versus THI response curves for B-2 and B-1 were related in a similar manner at both HW and MX. However, there was a peak effect of THI on CR for B-1 and B at MX but at B and B+1 at HW. A ready answer for these apparent discrepancies is not available. However, the differences may be due more to the mechanics of the test than real differences in the data. It is possible that they may be related to the time of service in relation to estrus at the two dairies. MX used mostly natural service, consequently cows were serviced in the morning or evening while in estrus. HW used artificial insemination and on most days serviced cows in the early afternoon.

The time of ovulation in relation to breeding can only be estimated. It is of interest because the authors have proposed that many of the events that led to depressed CR probably occurred prior to ovulation. Hall et al. (1959) proposed that during the warm season in Louisiana the average cow ovulated 12.4 hours after the end of estrus. If she was inseminated at the end of estrus at HW, ovulation might have occurred by midnight on the day of breeding. This would place the effect of B on CR prior to ovulation. The effect of B-2 on CR would have occurred 42 to 66 hours prior to ovulation or 24 to 48 hours before the beginning of estrus. This is the period of dramatic hormone concentration change during the cycle. Progesterone is in sharp decline, estrogen is rising sharply and LH, which has been showing a slow increase for several days is beginning to rise sharply (Hansel et al., 1973). According to the theories that have been proposed, the effect of heat stress at this time of the cycle (aside from a stress severe enough to cause direct damage to the ova) could result from

suppression of estrogen synthesis. At low estrogen concentrations, a weak estrus or complete failure to show estrus might result and the "ovulatory spurt" of LH might not materialize or be delayed.

Since the data from HW and MX came from cows that showed estrus and were serviced they must have had sufficient estrogen to show behavioral estrus. Therefore, another explanation for their problems must be found. Perhaps a critical concentration of estrogen and/or LH is necessary for the final maturation of the ova on the second and third day prior to breeding. With a depressed concentration of either one or both an imperfect ovum might be formed. Still another explanation might be that during this final maturation period the ova are more susceptible to damage by direct thermal stress.

Early embryo mortality      Considerable emphasis has been placed on the probability that early embryo mortality is an important aspect of the low fertility found in heat-stressed animals. Work with rabbits and sheep (Dutt, 1964; Ulberg and Burfening, 1967 and Thwaites, 1967), in which animals were slaughtered and eggs retrieved or embryos counted, substantiate this theory. However, the evidence for cows is mostly circumstantial. It is based on increased cycle length during the summer months for cows that return to service after having been inseminated. Stott and Williams (1962) proposed that heat stress caused increased embryo mortality and presented data indicating that the number of estrous cycles over 26 days was increased during the period from June to October in Arizona. They examined cows that did not return to estrus for pregnancy by rectal palpation at 35 to 42 days post-breeding. Having found a large number of

animals not pregnant they concluded that a high rate of embryonic loss occurred prior to 35 days. Two cows sacrificed at 35 days post-breeding contained embryonic membranes with evidence of degenerating embryos. However, irrespective of the validity of their conclusions, it is doubtful that two sets of membranes or an increased number of estrous cycles that extend beyond 26 days justifies a blanket diagnosis of increased early embryonic death. During the warm season estrus may be difficult to detect. Consequently, many estrous periods are missed, even under reasonably good management conditions.

It is generally accepted that, if an embryo dies after the time in the cycle that the CL would normally start to decline in function, the cycle length will be extended to between 26 and 34 days depending on the time of embryo death. With this in mind, an effort was made to determine if the percentage of intervals between services within this range actually increased during the warmer months (Figure 29). Although a significant increase in intervals between 26 and 34 days in length was found it was only 4.3% higher than in the cooler season. However, it does support the thesis that there is increased early embryo death during periods of heat stress.

#### Indirect effects of heat stress on fertility

Depressed pituitary gonadotropin      Madan and Johnson (1971)

have reported that plasma LH was depressed in cycling, heat stressed (THI 83.8) heifers. This gives support to the widely held hypothesis that the hypothalamus is depressed with a consequent depression of pituitary function during heat stress. They reported that both peak and baseline

levels of LH were significantly depressed and that the period between peaks was significantly longer in heat stressed heifers.

One expected result of depressed LH during the cycle would be reduced estrogen production since LH is considered necessary for its synthesis. If the LH stimulus to the ovary was depressed during a period of follicle growth when estrogen levels were critical, an imperfect, possibly RNA deficient, ovum might result. Such an ovum might survive to be fertilized but die during cell division because of an inability to synthesize sufficient new protein.

A second consequence of depressed estrogen production might be a reduced rate of follicle growth resulting in later maturity. Theoretically, it would take longer for the follicle to reach the state in which it could produce sufficient estrogen to "trigger" an ovulatory spurt of LH. An extended cycle length would be expected and in extreme cases of LH depression, estrous cycles would be absent due to inadequate stimulation for follicle growth. Support for this later thesis has been given in a report by Bond and McDowell (1972) indicating that winter acclimated heifers ceased cycling for an average of 16 weeks when placed in the climate chamber at a THI of 82.6. Rectal palpation of their ovaries indicated that they were inactive. The situation was diagnosed as one of depressed gonadotropin stimulation of the ovaries but LH levels were not available.

Plasma adrenal corticoids and thyroxine      The plasma adrenal corticoid concentrations and plasma thyroxine reported for cows at the Waialeale Experimental Farm (Experiment I) are probably applicable to HW

since both are north shore dairies. The slightly reduced values obtained during the warm season might help explain reduced milk production during the warm season, but assigning such changes a role in reduced fertility is difficult especially since experiments with thyroid damaged cattle and heat stressed, adrenalectomized animals have not resulted in depressed reproductive efficiency (Fernandez-Cano, 1958b; Howarth, 1969; and Tilton et al., 1972). Average daily THI during the experiment did not rise above 76. If THI had risen above 76, it is possible that a different hormonal picture would have evolved since the animals would have been forced to make more adjustments. Only if the plasma corticoid levels of animals in the field can be shown to increase rather than decrease, as has been reported for animals under a constant pattern of stress in the climate chamber (Christison and Johnson, 1972), will it be possible to build a thesis about reduced fertility from our present knowledge. If heat stress caused a significant sustained increase in ACTH, plasma corticoids or plasma progesterone it would be possible to construct a theory around their effects on the CL (Brunner et al., 1969 and Wagner et al., 1972). However, according to our present information these hormones are either depressed or do not change significantly.

Nutrition and CR      There was no indication that nutrition was involved in the depressed CR at either MX or HW. However, the ovaries of HW heifers that had been on pasture without sufficient supplementation (as determined by body condition) during the last trimester of pregnancy were often diagnosed as inactive at the 25 to 32 day post-calving examination. By 60 days post-calving most of their ovaries were diagnosed active.

Although a survey was not made, there was no obvious indication that these animals had a lower CR once they began to cycle and were inseminated.

As is the case with most high producing animals, many of the cows at HW were in a negative energy balance during early lactation. Blood samples taken at various times during the first 2 months of lactation indicated that hemoglobin levels tended to decline during the first 30 days post-calving but had returned to normal by 60 days post-calving. Wagner (1962) and Wagner and Hansel (1969) have implicated hemoglobin levels below 10 g/100 ml with anestrus and delayed ovarian activity in the postpartum period. These low hemoglobin levels (average 9.87 g/100 ml) at 30 days may help explain failure to detect estrus during the postpartum period at HW.

Post-calving uterine infection All of the reproductive problems of the dairy cows in Hawaii are not due to the THI of the cycle at which the cow is inseminated. In addition dairymen cull many cows after repeated services at several seasons of the year. Preliminary investigations indicate that the major cause of the problem is a post-calving infection that has resulted in some degree of endometritis. The organisms recovered have been non-specific bacteria. Alpha hemolytic streptococcus, staphylococcus epidermitis, micrococcus and E. coli have been recovered from most uteri.

The problem may in part have its origin in the climate since the weather is ideal for propagation of organisms throughout the year. Although HW cows calved in a pasture maintained for that purpose, 35 to 40% of the cows had some degree of endometritis detectable by speculum or palpation at the 30 day post-calving check. Once the infection was

established many cows were unable to eliminate the organisms completely without rather extensive treatment, even though the endometrial changes appeared to be minor. It is possible that depressed hormone systems resulting from heat stress were reducing the resistance of these tissues to infection. Estrogen is known to intensify acute leukocytic responses to infection in the uterus (Hawk, 1971). If it is depressed in heat stress as has been suggested, one result might be a reduced ability to eliminate postpartum uterine infections.



## CONCLUSIONS

In Experiment I, weather, milk production, rectal temperatures, plasma corticoid levels and plasma thyroxine were monitored at 2 to 3 week intervals using five shaded and four unshaded Holstein cows from September to December in a mildly heat stressing climate on Oahu, Hawaii. Average daily temperature-humidity index (THI) was negatively correlated with plasma corticoids in shaded cows, plasma thyroxine in both shaded and unshaded cows, milk production in unshaded cows and positively correlated with rectal temperatures.

Afternoon rectal temperatures were significantly elevated in the unshaded group but not in the shaded group indicating that the shades were effective in reducing thermal stress and that, within the average daily THI range of this experiment (70.0 to 75.2), afternoon THI per se did not add significantly to the heat stress of the cows. Both shaded and unshaded groups started each day with above normal rectal temperatures, i. e., they did not lose all of their excess heat load during the relatively warm nights. The elevated early morning temperatures that resulted contributed significantly to increased afternoon rectal temperatures.

Although unshaded cows gained more heat during the day, they lost heat more efficiently during the night, suggesting there was an advantage in being exposed to the night sky. However, this in itself was not enough to reduce their heat load to normal during the night. An evaluation of methods of cooling at night is suggested.

In the shaded group, plasma corticoid levels were higher and negatively correlated with THI. They were also negatively correlated with rectal temperature in both groups. However, during the experiment they were not depressed below levels reported as normal by other investigators.

The shaded group gave 3.7 kg/cow/day more milk than the unshaded group at THI 75. However, the design of this experiment and the small number of animals involved, caution against attributing all of this difference to shade treatment. Shaded cows also produced more milk fat and had higher milk fat percentages at THI above 74.

In summary, the results of Experiment I indicate that shade is beneficial for reducing heat stress in this climate and that this relief may be translated into increased productive efficiency. Further investigations will be necessary to determine the level of economic return that might be expected from shade in a commercial operation.

Experiment II was an evaluation of local weather data and herd breeding records for a 1300 Holstein dairy in Hawaii (HW) and a 600 cow Holstein dairy in Mexico (MX) to determine the effects of climate on reproductive performance. Climate caused a significant depression in conception rate (CR), especially at THI above 76. During 2 years in which THI did not rise above 76 at HW in Hawaii, warm season conception rates were above 50%. However, when average daily THI rose above 76, as they do in most years on leeward Oahu, Hawaii, CR were significantly depressed.

Conception rates improved at both HW and MX as cattle acclimated to higher THI, but CR were depressed during the period of acclimation. This indicates that the THI to which an animal is acclimated as well as the THI

to which it is exposed must be considered when evaluating effect of climate on CR.

It was established by several methods that CR was variably depressed by an increase in THI on individual days before breeding. Of the days evaluated, the THI of the second and third day prior to breeding affected CR most at both HW and MX. However, the average daily THI of each day from mid-estrous cycle till breeding was significantly correlated with CR at HW. Therefore, although CR were most sensitive to increased THI on the 3 days prior to breeding, these data indicate a complete THI-CR evaluation must also consider heat stress for over half of the estrous cycle preceding breeding. The pattern of CR response to THI supports the thesis that the ovum is most susceptible to damage during periods of rapid follicle growth.

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APPENDIX

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Table 101a. The temperature-humidity index (THI) formulas

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THI may be calculated from any of the following formulas depending on the form of the data.

1. Using degrees Fahrenheit.

$$\text{THI} = 0.55 \times t_d + 0.2t_{dp} + 17.5$$

$$\text{THI} = 0.4 (t_d + t_w) + 15$$

$$\text{THI} = t_d - 0.55 (1-\text{RH}) (t_d - 58)$$

2. Using degrees centigrade.

$$\text{THI} = C_d + .36 C_{dp} + 41.2$$

$$\text{THI} = 0.72 (C_d + C_w) + 40.6$$

Where:

$t_d$  = dry bulb temperature (F)

$t_w$  = wet bulb temperature (F)

$t_{dp}$  = dew point temperature (F)

RH = relative humidity (%  $\div$  100)

$C_d$  = dry bulb temperature (C)

$C_w$  = wet bulb temperature (C)

$C_{dp}$  = dew point temperature (C)

Table 101b. The temperature-humidity index (THI) - table of values

| °F | RELATIVE HUMIDITY % |      |      |      |      |      |      |      |      |      |      | °C   |
|----|---------------------|------|------|------|------|------|------|------|------|------|------|------|
|    | 50                  | 55   | 60   | 65   | 70   | 75   | 80   | 85   | 90   | 95   | 100  |      |
| 65 | 63.1                | 63.3 | 63.5 | 63.7 | 63.8 | 64.0 | 64.2 | 64.4 | 64.6 | 64.8 | 65.0 | 18.3 |
| 66 | 63.8                | 64.0 | 64.2 | 64.5 | 64.7 | 64.9 | 65.1 | 65.3 | 65.6 | 65.8 | 66.0 | 18.9 |
| 67 | 64.5                | 64.8 | 65.0 | 65.3 | 65.5 | 65.8 | 66.0 | 66.3 | 66.5 | 66.8 | 67.0 | 19.4 |
| 68 | 65.2                | 65.5 | 65.8 | 66.1 | 66.3 | 66.6 | 66.9 | 67.2 | 67.4 | 67.7 | 68.0 | 20.0 |
| 69 | 66.0                | 66.3 | 66.6 | 66.9 | 67.2 | 67.5 | 67.8 | 68.1 | 68.4 | 68.7 | 69.0 | 20.6 |
| 70 | 66.7                | 67.0 | 67.4 | 67.7 | 68.0 | 68.3 | 68.7 | 69.0 | 69.3 | 69.7 | 70.0 | 21.1 |
| 71 | 67.4                | 67.8 | 68.1 | 68.5 | 68.9 | 69.2 | 69.6 | 69.9 | 70.3 | 70.6 | 71.0 | 21.7 |
| 72 | 68.1                | 68.5 | 68.9 | 69.3 | 69.7 | 70.1 | 70.5 | 70.8 | 71.2 | 71.6 | 72.0 | 22.2 |
| 73 | 68.9                | 69.3 | 69.7 | 70.1 | 70.5 | 70.9 | 71.3 | 71.8 | 72.2 | 72.6 | 73.0 | 22.8 |
| 74 | 69.6                | 70.0 | 70.5 | 70.9 | 71.4 | 71.8 | 72.2 | 72.7 | 73.1 | 73.6 | 74.0 | 23.3 |
| 75 | 70.3                | 70.8 | 71.3 | 71.8 | 72.2 | 72.7 | 73.1 | 73.6 | 74.1 | 74.5 | 75.0 | 23.9 |
| 76 | 71.0                | 71.5 | 72.0 | 72.5 | 73.0 | 73.5 | 74.0 | 74.5 | 75.0 | 75.5 | 76.0 | 24.4 |
| 77 | 71.8                | 72.3 | 72.8 | 73.3 | 73.9 | 74.4 | 74.9 | 75.4 | 76.0 | 76.5 | 77.0 | 25.0 |
| 78 | 72.5                | 73.0 | 73.6 | 74.1 | 74.7 | 75.2 | 75.8 | 76.3 | 76.9 | 77.4 | 78.0 | 25.6 |
| 79 | 73.2                | 73.8 | 74.4 | 75.0 | 75.5 | 76.1 | 76.7 | 77.3 | 77.8 | 78.4 | 79.0 | 26.1 |
| 80 | 73.9                | 74.6 | 75.2 | 75.8 | 76.4 | 77.0 | 77.6 | 78.2 | 78.8 | 79.4 | 80.0 | 26.7 |
| 81 | 74.7                | 75.3 | 75.9 | 76.6 | 77.2 | 77.8 | 78.5 | 79.1 | 79.7 | 80.4 | 81.0 | 27.2 |
| 82 | 75.4                | 76.1 | 76.7 | 77.4 | 78.0 | 78.7 | 79.4 | 80.0 | 80.7 | 81.3 | 82.0 | 27.8 |
| 83 | 76.1                | 76.8 | 77.5 | 78.2 | 78.9 | 79.6 | 80.2 | 80.9 | 81.6 | 82.3 | 83.0 | 28.3 |
| 84 | 76.8                | 77.6 | 78.3 | 79.0 | 79.7 | 80.4 | 81.1 | 81.9 | 82.6 | 83.3 | 84.0 | 28.9 |
| 85 | 77.6                | 78.3 | 79.1 | 79.8 | 80.5 | 81.3 | 82.0 | 82.8 | 83.5 | 84.3 | 85.0 | 29.4 |
| 86 | 78.3                | 79.1 | 79.8 | 80.6 | 81.4 | 82.1 | 82.9 | 83.7 | 84.5 | 85.2 | 86.0 | 30.0 |
| 87 | 79.0                | 79.8 | 80.6 | 81.4 | 82.2 | 83.0 | 83.8 | 84.6 | 85.4 | 86.2 | 87.0 | 30.6 |
| 88 | 79.7                | 80.6 | 81.4 | 82.2 | 83.0 | 83.9 | 84.7 | 85.5 | 86.3 | 87.2 | 88.0 | 31.1 |
| 89 | 80.5                | 81.3 | 82.2 | 83.0 | 83.9 | 84.7 | 85.6 | 86.4 | 87.3 | 88.1 | 89.0 | 31.7 |
| 90 | 81.2                | 82.1 | 83.0 | 83.8 | 84.7 | 85.6 | 86.5 | 87.4 | 88.2 | 89.1 | 90.0 | 32.2 |

Table 102. Ambient temperature, relative humidity and resulting THI on blood collection days

| Time of Day     | Bleeding Day |             |            |            |            |           |            |            |
|-----------------|--------------|-------------|------------|------------|------------|-----------|------------|------------|
|                 | Sept.<br>13  | Sept.<br>26 | Oct.<br>10 | Oct.<br>29 | Nov.<br>14 | Dec.<br>5 | Dec.<br>30 | Feb.<br>10 |
| 0400            |              |             |            |            |            |           |            |            |
| Temperature (C) | 22.8         | 21.7        | 22.8       | 21.1       | 21.1       | 20.0      | 19.4       | 20.0       |
| Temperature (F) | 73           | 71          | 73         | 70         | 70         | 68        | 67         | 68         |
| Humidity        | 88           | 87          | 85         | 92         | 76         | 86        | 81         | 84         |
| THI             | 72           | 71          | 72         | 70         | 68         | 67        | 66         | 67         |
| 0800            |              |             |            |            |            |           |            |            |
| Temperature (C) | 22.8         | 21.1        | 22.8       | 21.7       | 21.1       | 17.2      | 20.6       | 18.3       |
| Temperature (F) | 73           | 70          | 73         | 71         | 70         | 63        | 69         | 65         |
| Humidity        | 86           | 88          | 85         | 89         | 76         | 99        | 65         | 91         |
| THI             | 72           | 69          | 72         | 70         | 68         | 63        | 67         | 65         |
| 1200            |              |             |            |            |            |           |            |            |
| Temperature (C) | 30.0         | 29.4        | 27.8       | 27.2       | 26.1       | 25.6      | 23.9       | 26.1       |
| Temperature (F) | 86           | 85          | 82         | 81         | 79         | 78        | 75         | 79         |
| Humidity        | 57           | 59          | 60         | 61         | 75         | 64        | 63         | 70         |
| THI             | 79           | 79          | 77         | 76         | 76         | 74        | 72         | 75         |
| 1600            |              |             |            |            |            |           |            |            |
| Temperature (C) | 30.0         | 28.9        | 27.8       | 26.7       | 25.6       | 25.0      | 23.3       | 25.6       |
| Temperature (F) | 86           | 84          | 82         | 80         | 78         | 77        | 74         | 78         |
| Humidity        | 57           | 59          | 62         | 65         | 70         | 70        | 66         | 71         |
| THI             | 79           | 78          | 77         | 76         | 75         | 74        | 71         | 75         |
| 2000            |              |             |            |            |            |           |            |            |
| Temperature (C) | 25.0         | 24.4        | 23.3       | 22.2       | 23.3       | 22.8      | 21.1       | 22.2       |
| Temperature (F) | 77           | 76          | 74         | 72         | 74         | 73        | 70         | 72         |
| Humidity        | 75           | 85          | 84         | 85         | 82         | 79        | 79         | 80         |
| THI             | 74           | 74          | 73         | 71         | 72         | 72        | 67         | 70         |
| Average         | 75.2         | 74.2        | 74.2       | 72.6       | 71.8       | 70.0      | 69.0       | 70.4       |

Table 103. Rectal temperatures (C) of individual cows at the time plasma samples were taken

|                 |      | Unshaded Cow Number |       |       |       |                  | Shaded Cow Number |       |       |       |       |                  |
|-----------------|------|---------------------|-------|-------|-------|------------------|-------------------|-------|-------|-------|-------|------------------|
|                 | THI  | 550                 | 586   | 643   | 648   | Mean $\pm$ SD    | 600               | 608   | 633   | 637   | 646   | Mean $\pm$ SD    |
| <b>Sept. 13</b> |      |                     |       |       |       |                  |                   |       |       |       |       |                  |
| 0400            | 72   | 38.89               | 38.89 | 38.94 | 40.11 | 39.21 $\pm$ 0.50 | 39.28             | ----- | 39.56 | 39.00 | ----- | 39.28 $\pm$ 0.28 |
| 0800            | 72   | 39.00               | 38.61 | 38.72 | 39.56 | 38.97 $\pm$ 0.42 | 38.72             | ----- | 39.39 | 39.28 | ----- | 39.13 $\pm$ 0.36 |
| 1200            | 79   | 39.61               | 38.94 | 38.89 | 40.39 | 39.45 $\pm$ 0.70 | 39.11             | 38.89 | 39.72 | 39.33 | 38.94 | 39.20 $\pm$ 0.34 |
| 1600            | 79   | 39.56               | 39.56 | 39.89 | 40.67 | 39.92 $\pm$ 0.52 | 39.06             | 39.00 | 39.56 | 39.39 | 38.94 | 39.19 $\pm$ 0.27 |
| 2000            | 74   | 39.22               | 39.11 | 39.22 | 39.78 | 39.33 $\pm$ 0.30 | 38.89             | 38.94 | 39.22 | 39.17 | 39.00 | 39.04 $\pm$ 0.14 |
| Avg.            | 75.2 | 39.26               | 39.02 | 39.13 | 40.10 | 39.38 $\pm$ 0.49 | 39.01             | 38.94 | 39.49 | 39.23 | 38.96 | 39.13 $\pm$ 0.23 |
| <b>Sept. 26</b> |      |                     |       |       |       |                  |                   |       |       |       |       |                  |
| 0400            | 71   | 39.11               | 39.11 | 38.83 | 40.06 | 39.28 $\pm$ 0.54 | 39.28             | 39.06 | 39.44 | 39.67 | 39.56 | 39.40 $\pm$ 0.24 |
| 0800            | 69   | 39.22               | 39.17 | 38.89 | 40.06 | 39.33 $\pm$ 0.50 | 39.28             | 38.83 | 39.72 | 39.33 | 39.56 | 39.44 $\pm$ 0.34 |
| 1200            | 79   | 39.55               | 39.22 | 39.55 | 40.22 | 39.63 $\pm$ 0.42 | 39.50             | 38.89 | 39.89 | 39.33 | 39.27 | 39.38 $\pm$ 0.36 |
| 1600            | 78   | 40.39               | 40.28 | 39.94 | 41.17 | 40.44 $\pm$ 0.52 | 39.56             | 39.17 | 40.83 | 39.44 | 39.67 | 39.73 $\pm$ 0.64 |
| 2000            | 74   | 39.56               | 39.44 | 39.56 | 40.44 | 39.75 $\pm$ 0.46 | 39.17             | 39.00 | 40.44 | 39.22 | 39.56 | 39.48 $\pm$ 0.57 |
| Avg.            | 74.2 | 39.57               | 39.44 | 39.35 | 40.39 | 39.69 $\pm$ 0.48 | 39.36             | 38.99 | 40.06 | 39.40 | 39.52 | 39.47 $\pm$ 0.39 |
| <b>Oct. 10</b>  |      |                     |       |       |       |                  |                   |       |       |       |       |                  |
| 0400            | 72   | 39.44               | 38.72 | 38.56 | 39.61 | 39.08 $\pm$ 0.52 | 38.78             | 38.78 | 39.33 | 38.89 | 38.78 | 38.91 $\pm$ 0.24 |
| 0800            | 72   | 39.17               | 38.61 | 38.56 | 40.11 | 39.11 $\pm$ 0.72 | 39.22             | 38.94 | 39.83 | 39.33 | 39.00 | 39.26 $\pm$ 0.35 |
| 1200            | 77   | 39.44               | 38.94 | 38.83 | 40.28 | 39.37 $\pm$ 0.66 | 38.56             | 38.50 | 39.00 | 38.67 | 38.78 | 38.70 $\pm$ 0.20 |
| 1600            | 77   | 40.22               | 40.33 | 40.22 | 41.39 | 40.54 $\pm$ 0.57 | 39.33             | 38.89 | 39.67 | 39.61 | 39.72 | 39.44 $\pm$ 0.34 |
| 2000            | 73   | 39.11               | 38.94 | 39.00 | 40.06 | 39.28 $\pm$ 0.53 | 39.33             | 39.00 | 38.89 | 39.28 | 38.94 | 39.09 $\pm$ 0.20 |
| Avg.            | 74.2 | 39.48               | 39.11 | 39.03 | 40.29 | 39.48 $\pm$ 0.58 | 39.04             | 38.82 | 39.34 | 39.16 | 39.04 | 39.08 $\pm$ 0.19 |
| <b>Oct. 29</b>  |      |                     |       |       |       |                  |                   |       |       |       |       |                  |
| 0400            | 70   | 38.17               | 38.89 | 38.33 | 39.72 | 38.78 $\pm$ 0.70 | 38.89             | 38.67 | 39.11 | 39.06 | 39.22 | 38.99 $\pm$ 0.21 |
| 0800            | 70   | 38.56               | 38.61 | 38.50 | 39.83 | 38.37 $\pm$ 0.64 | 38.78             | 38.78 | 39.17 | 39.17 | 39.11 | 39.00 $\pm$ 0.20 |
| 1200            | 76   | 39.89               | 39.11 | 39.22 | 40.11 | 39.58 $\pm$ 0.49 | 39.00             | 38.67 | 39.39 | 39.67 | 38.89 | 39.12 $\pm$ 0.40 |
| 1600            | 76   | 40.22               | 40.00 | 39.78 | 40.83 | 40.21 $\pm$ 0.45 | 39.22             | 38.94 | 39.39 | 40.22 | 39.33 | 39.42 $\pm$ 0.48 |
| 2000            | 71   | 40.11               | 39.11 | 39.44 | 40.56 | 39.30 $\pm$ 0.65 | 38.89             | 39.28 | 39.67 | 39.56 | 39.72 | 39.42 $\pm$ 0.34 |
| Avg.            | 72.6 | 39.39               | 39.14 | 39.05 | 40.21 | 39.44 $\pm$ 0.53 | 38.96             | 38.87 | 39.35 | 39.54 | 39.25 | 39.19 $\pm$ 0.28 |



Table 103. (continued)

|         |      | Unshaded Cow Number |       |       |       |            | Shaded Cow Number |       |       |       |       |            |
|---------|------|---------------------|-------|-------|-------|------------|-------------------|-------|-------|-------|-------|------------|
|         | THI  | 550                 | 586   | 643   | 648   | Mean±SD    | 600               | 608   | 633   | 637   | 646   | Mean±SD    |
| Nov. 14 |      |                     |       |       |       |            |                   |       |       |       |       |            |
| 0400    | 68   | 39.00               | 38.76 | 38.78 | 39.33 | 38.97±0.26 | 39.22             | 38.89 | 39.56 | 39.00 | 38.78 | 39.09±0.31 |
| 0800    | 68   | 38.78               | 38.78 | 38.44 | 39.33 | 38.83±0.37 | 38.44             | 38.33 | 39.22 | 39.11 | 38.78 | 38.73±0.39 |
| 1200    | 76   | 39.83               | 39.56 | 39.17 | 40.11 | 39.67±0.40 | 38.94             | 38.89 | 39.50 | 39.28 | 38.89 | 39.10±0.28 |
| 1600    | 75   | 39.33               | 39.89 | 39.44 | 40.28 | 39.73±0.44 | 38.89             | 38.83 | 39.27 | 38.83 | 39.28 | 39.02±0.23 |
| 2000    | 72   | 39.44               | 38.94 | 39.33 | 40.44 | 39.54±0.54 | 39.28             | 39.11 | 39.61 | 39.17 | 39.11 | 39.26±0.21 |
| Avg.    | 71.8 | 39.28               | 39.19 | 39.03 | 39.90 | 39.35±0.38 | 38.95             | 38.81 | 39.43 | 39.08 | 38.97 | 39.05±0.24 |
| Dec. 5  |      |                     |       |       |       |            |                   |       |       |       |       |            |
| 0400    | 67   | -----               | ----- | ----- | ----- | -----      | -----             | ----- | ----- | ----- | ----- | -----      |
| 0800    | 63   | -----               | ----- | ----- | ----- | -----      | -----             | ----- | ----- | ----- | ----- | -----      |
| 1200    | 74   | -----               | ----- | ----- | ----- | -----      | -----             | ----- | ----- | ----- | ----- | -----      |
| 1600    | 74   | 38.89               | 39.44 | 38.44 | 39.44 | 39.05±0.48 | 39.11             | 38.44 | 39.33 | 38.89 | 38.67 | 38.89±0.35 |
| 2000    | 72   | 38.72               | 39.33 | 38.78 | 38.89 | 38.92±0.28 | 38.78             | 38.89 | 39.67 | 38.94 | 38.89 | 39.02±0.36 |
| Avg.    | 70.0 | 38.80               | 39.38 | 38.61 | 39.16 | 38.90±0.35 | 38.94             | 38.66 | 39.50 | 38.91 | 38.78 | 38.96±0.32 |
| Dec. 30 |      |                     |       |       |       |            |                   |       |       |       |       |            |
| 0400    | 66   | 38.50               | 38.33 | 38.56 | 38.56 | 38.40±.11  | 38.56             | 38.56 | 38.89 | 39.17 | 39.22 | 38.83±.32  |
| 0800    | 67   | 38.83               | 38.33 | 38.28 | 38.67 | 38.53±.27  | 38.28             | 38.44 | 38.78 | 38.44 | 39.00 | 38.59±.29  |
| 1200    | 72   | 38.68               | 38.17 | 38.33 | 38.61 | 38.45±.24  | 38.28             | 38.33 | 38.94 | 39.11 | 39.11 | 38.75±.42  |
| 1600    | 71   | 38.83               | 38.72 | 38.89 | 38.89 | 38.83±.08  | 38.39             | ----- | ----- | 39.44 | 39.56 | 39.13±.64  |
| 2000    | 67   | 38.78               | 38.67 | 38.67 | 39.56 | 38.92±.42  | 40.06             | ----- | ----- | 39.72 | 39.50 | 39.76±.28  |
| Avg.    | 69.0 | 38.72               | 38.44 | 38.55 | 38.86 | 38.64±.18  | 38.71             | 38.44 | 38.87 | 39.18 | 39.28 | 38.90±.34  |
| Feb. 10 |      |                     |       |       |       |            |                   |       |       |       |       |            |
| 0400    | 67   | 38.78               | ----- | ----- | 38.44 | 38.61±.24  | -----             | ----- | 38.33 | 38.33 | 38.89 | 38.52±.32  |
| 0800    | 65   | 38.22               | ----- | ----- | 38.33 | 38.27±.08  | 38.44             | ----- | 38.22 | 38.44 | 38.67 | 38.44±.18  |
| 1200    | 75   | 38.44               | ----- | ----- | 39.00 | 38.72±.40  | 38.67             | ----- | 38.61 | 39.06 | 39.72 | 39.01±.51  |
| 1600    | 75   | 39.44               | ----- | ----- | 39.44 | 39.44±.00  | 39.00             | ----- | 39.33 | 40.17 | 41.00 | 39.87±.90  |
| 2000    | 70   | 38.83               | ----- | ----- | 38.67 | 38.79±.11  | 38.33             | ----- | 38.39 | 38.67 | 39.33 | 38.68±.46  |
| Avg.    | 70.4 | 38.74               | ----- | ----- | 38.78 | 38.76±.03  | 38.61             | ----- | 38.58 | 38.93 | 39.52 | 38.91±.44  |

Table 104. Plasma corticoid assay

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1. Pipet 0.2 ml aliquots of plasma or cortisol standard into 16 x 125 screw cap disposable glass culture tubes. Standards were 0, 6, 12, 24, 36, 48 and 60 mg/ml cortisol prepared in distilled water.
  2. Add 5 ml of nanograde hexane<sup>1</sup> to each tube.
  3. Shake tubes manually for 30 seconds.
  4. Centrifuge for 10 minutes to separate the contents into two phases.
  5. Place tubes in a dry ice-alcohol bath until plasma has frozen at the bottom of the tubes.
  6. Pour off and discard hexane.
  7. Place tubes in a 45 C water bath to thaw plasma.
  8. Add 4.5 ml nanograde methylene chloride<sup>1</sup> to each tube, shake and centrifuge in the same manner as above.
  9. Aspirate plasma (water phase) from top of solvent.
  10. Transfer a 1 ml aliquot of the methylene chloride extract to each of two 12 x 75 mm disposable glass culture tubes (unknowns) or each of 3 tubes (standards).
  11. Evaporate methylene chloride in a 45 C water bath.
  12. Add 1 ml of corticoid-binding globulin (CBG) - <sup>3</sup>H - cortisol solution (Appendix Table 105a).
  13. Incubate tubes for 5 minutes in 45 C water bath followed by 15 minutes in a 10 C water bath.
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<sup>1</sup> Mallinckrodt Chemical Works.

Table 104. (continued)

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14. Add 40 mg of florisil<sup>2</sup>, mix slowly on a vortex mixer for 30 seconds and return to 10 C water bath for 5 minutes.
  15. Transfer a 0.5 ml aliquot of the supernatant to a scintillation vial for counting.
  16. Add 10 ml of scintillation fluid (Appendix Table 105b) to vial and mix.
  17. Counts were taken in a Beckman LS-100 counter. Average time required to count each standard and unknown to a preset total count was determined.
  18. Data from the standards were used to determine the slope of the regression line which was used to calculate the corticoid concentrations in the unknowns with the following equation:

$$Y_i - \bar{y} = b(X_i - \bar{x})$$

This equation was solved for  $X_i$  (concentration of corticoid in unknown sample) as follows.

$$X_i = \frac{Y_i - \bar{y} + b\bar{x}}{b}$$

The terms used were defined as follows:

$Y_i$  = Time required to count the unknown

$b$  = Slope of the standard curve

$X_i$  = Corticoid (ng/ml) in unknown

$\bar{x}$  = Mean corticoid (ng/ml) for all standards

$\bar{y}$  = Mean time required to count standards

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<sup>2</sup>Fisher Scientific Co.

Table 105a. Preparation of corticoid-binding globulin (CBG) solution

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CBG Solution for Corticoid Assay. (For 100 ml CBG)

1. Place 4 ml of stock solution of  $^3\text{H}$ -cortisol in a round bottom flask (RBF) and evaporate the ethyl alcohol solvent on a rotary evaporator in a 45 C water bath.
  2. Rinse the flask with 1 ml of ethyl alcohol to resuspend the labelled corticoid.
  3. Add 96 ml of triple distilled water.
  4. Finally, add 3 ml of dog plasma. (Plasma is from a dog adrenal-ectomized 36-48 hours prior to blood collection).
  5. Mix by gentle manual rotation.
  6. Store at 4 C for at least 1 hour before use.
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Table 105b. Preparation of scintillation fluid

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Scintillation Fluid.

1. Place 5 g of diphenyloxazole (PPO) into a large bottle.
  2. Add 1 liter toluene.
  3. Add 100 ml Beckman Biosolv #3.
  4. Mix thoroughly.
-

Table 106. Plasma adrenal corticoid levels (ng/ml) by cow and bleeding

|          |      | Unshaded Cow Number |      |      |      |           | Shaded Cow Number |      |      |      |      |           |
|----------|------|---------------------|------|------|------|-----------|-------------------|------|------|------|------|-----------|
|          | THI  | 550                 | 586  | 643  | 648  | Mean±SD   | 600               | 608  | 633  | 637  | 646  | Mean±SD   |
| Sept. 13 |      |                     |      |      |      |           |                   |      |      |      |      |           |
| 0400     | 72   | 15.9                | 23.3 | 8.2  | ---- | 15.8± 7.5 | 21.3              | ---- | 6.8  | 18.0 | ---- | 15.4± 7.6 |
| 0800     | 72   | 12.6                | 23.6 | 10.8 | ---- | 15.7± 6.9 | 7.1               | ---- | 4.8  | 19.7 | ---- | 10.5± 8.0 |
| 1200     | 79   | 13.3                | 17.9 | 9.5  | ---- | 13.6± 4.2 | 8.3               | ---- | 7.7  | 23.5 | ---- | 13.2± 9.0 |
| 1600     | 79   | 11.6                | 7.7  | 24.6 | ---- | 14.6± 8.8 | 8.7               | ---- | 4.2  | 14.5 | ---- | 9.1± 5.2  |
| 2000     | 74   | 14.9                | 16.7 | 7.4  | ---- | 13.0± 4.9 | 3.7               | ---- | 5.1  | 5.7  | ---- | 4.8± 1.0  |
| Avg.     | 75.2 | 13.7                | 17.8 | 12.1 | ---- | 14.5± 2.9 | 9.8               | ---- | 5.7  | 16.3 | ---- | 10.6± 5.3 |
| Sept. 26 |      |                     |      |      |      |           |                   |      |      |      |      |           |
| 0400     | 71   | 7.5                 | 13.3 | 21.2 | 6.7  | 12.2± 6.7 | 3.9               | 23.2 | 7.2  | 0.0  | 10.6 | 9.0± 8.9  |
| 0800     | 69   | 7.2                 | 18.7 | 1.3  | 12.4 | 9.9± 7.4  | 10.4              | 10.7 | 11.2 | 0.1  | 4.6  | 7.4± 4.9  |
| 1200     | 79   | 3.2                 | 0.1  | 5.3  | 6.1  | 3.6± 2.6  | 8.6               | 12.3 | 3.1  | 4.7  | 5.9  | 6.9± 3.6  |
| 1600     | 78   | 8.9                 | 14.0 | 10.1 | 16.3 | 12.3± 3.4 | 3.2               | 7.0  | 11.9 | 28.8 | 17.8 | 13.7±10.0 |
| 2000     | 74   | 6.0                 | 0.1  | 0.0  | 1.5  | 1.9± 2.8  | 3.9               | 1.0  | 7.4  | 2.4  | 3.5  | 3.6± 2.4  |
| Avg.     | 74.2 | 6.6                 | 9.2  | 7.6  | 8.6  | 8.0± 1.1  | 6.0               | 10.8 | 8.2  | 7.2  | 8.5  | 8.1± 1.8  |
| Oct. 10  |      |                     |      |      |      |           |                   |      |      |      |      |           |
| 0400     | 72   | 10.9                | 0.9  | 20.8 | 7.2  | 9.9± 8.3  | 0.0               | 13.0 | 17.8 | 16.6 | 22.5 | 14.0± 8.5 |
| 0800     | 72   | 14.1                | 2.2  | 23.0 | 14.6 | 13.5± 8.6 | 6.1               | 7.6  | 16.9 | 13.1 | 3.9  | 10.5± 6.5 |
| 1200     | 77   | 0.4                 | 6.7  | 12.3 | 19.1 | 9.6± 8.0  | 2.6               | 11.0 | 2.6  | 2.9  | 6.1  | 5.0± 3.6  |
| 1600     | 77   | 9.5                 | 16.0 | 21.7 | 4.0  | 12.3± 7.7 | 2.4               | 8.2  | 11.5 | 26.3 | 29.2 | 15.5±11.7 |
| 2000     | 73   | 5.0                 | 2.5  | 12.7 | 8.4  | 7.1± 4.4  | 10.2              | 7.8  | 4.7  | 35.6 | 20.6 | 15.7±12.6 |
| Avg.     | 74.2 | 8.0                 | 5.7  | 13.1 | 10.7 | 10.6± 5.4 | 4.3               | 9.5  | 10.7 | 19.9 | 16.5 | 12.2± 6.1 |
| Oct. 29  |      |                     |      |      |      |           |                   |      |      |      |      |           |
| 0400     | 70   | 5.1                 | 16.1 | 43.5 | 3.4  | 13.3±20.9 | 26.6              | 3.3  | 24.6 | 16.6 | 20.1 | 18.2± 9.2 |
| 0800     | 70   | 1.0                 | 23.3 | 9.4  | 6.6  | 10.1± 9.5 | 13.5              | 3.4  | 11.6 | 18.1 | 10.0 | 11.3± 5.4 |
| 1200     | 76   | 1.9                 | 17.1 | 9.3  | 1.4  | 7.4± 7.4  | 15.1              | 3.3  | 14.1 | 2.9  | 8.9  | 8.9± 5.8  |
| 1600     | 76   | 5.4                 | 14.8 | 14.5 | 12.8 | 11.9± 4.4 | 33.1              | 3.6  | 29.5 | 26.3 | 21.1 | 22.7±11.6 |
| 2000     | 71   | 6.2                 | 6.5  | 29.0 | 4.7  | 11.6±11.6 | 16.1              | 3.7  | 30.8 | 35.6 | 28.2 | 22.8±12.9 |
| Avg.     | 72.6 | 3.9                 | 15.6 | 22.1 | 5.8  | 11.8± 8.5 | 20.9              | 3.5  | 22.1 | 19.9 | 17.7 | 16.8± 7.6 |

Table 106. (continued)

|           |      | Unshaded Cow Number |       |       |      |           | Shaded Cow Number |       |       |      |      |           |  |
|-----------|------|---------------------|-------|-------|------|-----------|-------------------|-------|-------|------|------|-----------|--|
| THI       |      | 550                 | 586   | 643   | 648  | Mean±SD   | 600               | 608   | 633   | 637  | 647  | Mean±SD   |  |
| Nov. 14   |      |                     |       |       |      |           |                   |       |       |      |      |           |  |
| 0400      | 68   | 0.8                 | 14.6  | 42.9  | 22.2 | 20.1±17.6 | 6.6               | 27.6  | 8.0   | 28.3 | 20.3 | 18.2±10.4 |  |
| 0800      | 68   | 8.2                 | 19.5  | 17.7  | 5.7  | 12.8± 6.8 | 8.0               | 20.3  | 3.1   | 30.6 | 15.7 | 15.5±10.7 |  |
| 1200      | 76   | 7.6                 | 15.0  | 11.2  | 2.7  | 9.1± 5.2  | 4.0               | 15.7  | 13.4  | 28.7 | 23.4 | 17.0± 9.5 |  |
| 1600      | 75   | 2.9                 | 16.9  | 33.4  | 16.5 | 17.4±12.5 | 8.9               | 9.3   | 16.1  | 8.5  | 14.6 | 11.5± 3.6 |  |
| 2000      | 72   | 11.1                | 17.3  | 32.1  | 14.2 | 18.7± 9.3 | 5.3               | 18.8  | 6.5   | 31.4 | 13.1 | 15.0±10.6 |  |
| Avg.      | 71.8 | 6.1                 | 16.7  | 27.5  | 12.3 | 15.6± 9.0 | 6.6               | 18.3  | 9.4   | 25.5 | 17.4 | 15.4± 7.5 |  |
| Dec. 5    |      |                     |       |       |      |           |                   |       |       |      |      |           |  |
| 0400      | 67   | 11.2                | 10.8  | 10.7  | 7.4  | 10.8± 1.8 | 9.7               | 7.2   | 76.0  | 36.7 | 48.0 | 35.5±28.6 |  |
| 0800      | 63   | 33.7                | 10.7  | 10.5  | 6.9  | 15.4±12.3 | 9.0               | 19.7  | 60.0  | 6.0  | 14.7 | 21.9±21.6 |  |
| 1200      | 74   | 24.0                | 7.5   | 19.7  | 10.1 | 15.3± 7.8 | 7.5               | 30.5  | 7.4   | 12.1 | 70.6 | 25.6±26.9 |  |
| 1600      | 74   | 3.5                 | 16.2  | 8.5   | 9.6  | 9.4± 5.2  | 5.0               | 29.5  | 4.8   | 55.6 | 63.0 | 31.6±27.3 |  |
| 2000      | 72   | 11.1                | 12.1  | 11.4  | Clot | 11.5± 0.5 | 5.9               | Clot  | 4.3   | 13.0 | 12.1 | 8.8± 4.4  |  |
| Avg.      | 70   | 16.7                | 11.5  | 12.2  | 8.5  | 12.2± 3.4 | 7.4               | 21.7  | 30.5  | 24.7 | 41.7 | 25.2±12.5 |  |
| Dec. 30   |      |                     |       |       |      |           |                   |       |       |      |      |           |  |
| 0400      | 66   | 13.4                | 2.8   | 20.9  | 8.0  | 11.3± 7.7 | 12.6              | ----- | 0.3   | 8.0  | 8.0  | 7.2± 5.1  |  |
| 0800      | 67   | 14.7                | 13.8  | 9.2   | 1.9  | 9.9± 5.9  | 20.0              | ----- | ----- | 9.0  | 1.5  | 10.2± 9.3 |  |
| 1200      | 72   | 2.4                 | 5.8   | 1.7   | 13.6 | 5.9± 5.6  | 5.3               | ----- | ----- | 10.0 | 0.3  | 5.2± 4.9  |  |
| 1600      | 71   | 14.4                | 5.2   | 13.2  | 6.6  | 9.8± 4.6  | 5.2               | ----- | ----- | 16.8 | 7.4  | 9.8± 6.2  |  |
| 2000      | 67   | 10.4                | 16.1  | 12.4  | 13.6 | 13.1± 2.4 | 2.6               | ----- | ----- | 20.0 | 7.6  | 10.1± 9.0 |  |
| Avg.      | 68.6 | 11.6                | 8.7   | 11.5  | 8.7  | 10.1± 1.6 | 9.1               | ----- | ----- | 12.8 | 5.0  | 8.9± 3.9  |  |
| Feb. 9-10 |      |                     |       |       |      |           |                   |       |       |      |      |           |  |
| 2000      | 71   | 7.3                 | ----- | ----- | 9.9  | 8.6± 1.8  | 10.6              | ----- | 28.6  | 12.2 | 29.1 | 20.1±10.1 |  |
| 0400      | 67   | 5.0                 | ----- | ----- | 12.2 | 8.6± 5.0  | 11.1              | ----- | 23.1  | 9.7  | 24.5 | 17.1± 7.7 |  |
| 0800      | 65   | 4.9                 | ----- | ----- | 4.5  | 4.7± 0.3  | 7.1               | ----- | 12.5  | 3.3  | 22.6 | 11.4± 8.4 |  |
| 1200      | 75   | 6.2                 | ----- | ----- | 4.7  | 5.4± 1.1  | 5.9               | ----- | 25.5  | 11.4 | 7.0  | 12.4± 9.0 |  |
| 1600      | 75   | 6.0                 | ----- | ----- | 8.9  | 7.4± 2.0  | 11.2              | ----- | 23.5  | 10.0 | 14.6 | 14.8± 6.1 |  |
| Avg.      | 70.6 | 5.9                 | ----- | ----- | 8.0  | 6.9± 1.5  | 9.2               |       | 22.6  | 9.3  | 19.6 | 15.2± 6.9 |  |

Table 107. Res-O-Mat ETR Diagnostic Test<sup>1</sup>

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Standard Serum Preparation

Volumetrically reconstitute the Standard Serum with 2 ml of distilled water. Allow to stand 5 to 10 minutes with periodic end-over-end mixing until the dried serum is completely dissolved and thoroughly mixed. Do not shake vigorously.

Test Procedure

The following directions are for performing the Res-O-Mat ETR Test on one unknown serum sample. The same procedure should be followed for each unknown serum and Standard Serum.

1. Volumetrically transfer 1 ml of serum into 2 ml of extraction alcohol in a 15 ml conical centrifuge tube. Cap or stopper the tube to prevent evaporation.
2. Mix the serum-alcohol mixture from Step 1 on a high-speed vortex mixer for approximately 15 seconds.
3. Centrifuge the mixture until the precipitated protein has been firmly packed at the bottom of the tube (2500 rpm for 5 minutes).
4. Volumetrically transfer 0.3 ml of the alcoholic supernatant to a labeled Res-O-Mat ETR Solution Vial.
5. Using forceps, fill a Serum Micropipet with the serum by placing the micropipet to a 45° angle or less and touching it to the serum. Observe closely to assure complete filling of the micropipet.
6. Using forceps, add one Res-O-Mat Strip to the solution vial and cap tightly.

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<sup>1</sup>Mallinckrodt Chemical Works, St. Louis, Missouri.

Table 107. (continued)

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7. Rotate the solution vial for 1 hour at 12 to 14 rpm on a rotator which provides an end-over-end mixing action.
  8. After the rotation period, remove the Res-O-Mat Strip with forceps. Dispose of the strip.
  9. Transfer 0.5 ml of solution to a scintillation vial.
  10. Add 10 ml of scintillation fluid (Appendix Table 105b) and mix.
  11. Count radioactivity.

$$\text{Effective Thyroxine Ratio (ETR)} = \frac{\text{Mean CPM of 2 Standards}}{\text{CMP of Unknown}}$$

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Table 108. Effective thyroxine ratio (ETR) values for individual samples

|          | THI  | Unshaded Cow Number |      |      |       |           | Shaded Cow Number |      |      |      |       | Mean±SD   |
|----------|------|---------------------|------|------|-------|-----------|-------------------|------|------|------|-------|-----------|
|          |      | 550                 | 586  | 643  | 648   | Mean±SD   | 600               | 608  | 633  | 637  | 646   |           |
| Sept. 26 |      |                     |      |      |       |           |                   |      |      |      |       |           |
| 0400     | 71   | .885                | .845 | .886 | .905  | .880±.025 | .900              | .914 | .871 | .919 | .987  | .918±.043 |
| 1600     | 78   | .857                | .834 | .865 | .885  | .860±.018 | .849              | .847 | .878 | .868 | .899  | .868±.019 |
| Avg.     | 74.2 | .871                | .839 | .875 | .895  | .870±.023 | .874              | .880 | .874 | .893 | .943  | .893±.029 |
| Oct. 10  |      |                     |      |      |       |           |                   |      |      |      |       |           |
| 0400     | 72   | .898                | .949 | .954 | .929  | .932±.025 | .920              | .903 | .889 | .919 | .951  | .916±.023 |
| 1600     | 77   | .886                | .897 | .902 | .883  | .892±.061 | .951              | .884 | .914 | .909 | .937  | .919±.026 |
| Avg.     | 74.2 | .892                | .923 | .928 | .906  | .912±.016 | .935              | .893 | .901 | .914 | .944  | .917±.022 |
| Oct. 29  |      |                     |      |      |       |           |                   |      |      |      |       |           |
| 0400     | 70   | .918                | .901 | .933 | .922  | .918±.013 | .849              | .885 | .919 | .906 | ----  | .890±.030 |
| 1600     | 76   | .888                | .896 | .932 | .998  | .928±.050 | .906              | .919 | .921 | .894 | .877  | .903±.018 |
| Avg.     | 72.6 | .903                | .898 | .932 | .960  | .923±.029 | .877              | .902 | .920 | .900 | .877  | .895±.018 |
| Nov. 14  |      |                     |      |      |       |           |                   |      |      |      |       |           |
| 0400     | 68   | .928                | .939 | .909 | .937  | .928±.014 | .893              | .954 | .916 | .929 | .937  | .926±.023 |
| 1600     | 75   | .941                | .941 | .961 | .947  | .947±.009 | ----              | .937 | .896 | .932 | .975  | .935±.032 |
| Avg.     | 71.8 | .934                | .940 | .935 | .942  | .938±.004 | .893              | .945 | .906 | .930 | .956  | .926±.026 |
| Dec. 5   |      |                     |      |      |       |           |                   |      |      |      |       |           |
| 0400     | 67   | .930                | .941 | .950 | .992  | .953±.027 | .883              | ---- | .860 | .924 | .992  | .915±.058 |
| 1600     | 74   | .906                | ---- | .926 | 1.000 | .944±.049 | .925              | .963 | .958 | .931 | 1.028 | .961±.041 |
| Avg.     | 70.0 | .918                | .941 | .938 | .996  | .948±.033 | .904              | .963 | .909 | .927 | 1.010 | .943±.044 |
| Avg.     |      | .904                | .905 | .922 | .940  | .918      | .897              | .912 | .902 | .913 | .954  | .915      |

Table 109. Milk production (kg) by cow and sampling day

| Date  | 550   | Unshaded Cow Numbers |       |       |                 | 600   | Shaded Cow Numbers |       |       |       | Mean            |
|-------|-------|----------------------|-------|-------|-----------------|-------|--------------------|-------|-------|-------|-----------------|
|       |       | 586                  | 643   | 648   | Mean            |       | 608                | 633   | 637   | 646   |                 |
| 9/5   | 21.36 | 11.45                | 15.45 | 14.32 | 15.64 $\pm$ 4.2 | 22.50 | 19.19              | 19.32 | 16.36 | 23.86 | 20.25 $\pm$ 3.0 |
| 9/26  | 22.56 | 13.18                | 17.73 | 12.50 | 16.50 $\pm$ 4.7 | 21.59 | 16.36              | 18.86 | 11.82 | 26.81 | 19.11 $\pm$ 5.6 |
| 10/10 | 18.86 | 11.00                | 14.64 | 11.82 | 14.08 $\pm$ 3.5 | 20.54 | 17.05              | 19.32 | 14.77 | 22.82 | 18.90 $\pm$ 3.1 |
| 10/29 | 14.45 | 10.68                | 16.14 | 13.55 | 13.71 $\pm$ 2.3 | 21.81 | 13.27              | 17.27 | 12.64 | 21.68 | 17.35 $\pm$ 4.4 |
| 11/14 | 15.14 | 10.59                | 16.27 | 12.95 | 13.74 $\pm$ 2.5 | 21.36 | 14.19              | 20.23 | 16.23 | 22.27 | 18.86 $\pm$ 3.5 |
| 12/5  | 15.45 | 9.18                 | 17.05 | 12.05 | 13.43 $\pm$ 3.5 | 18.05 | 10.91              | 17.95 | 15.23 | 20.91 | 16.61 $\pm$ 3.8 |
| Avg.  | 17.97 | 11.01                | 16.21 | 12.86 | 14.52 $\pm$ 3.1 | 20.99 | 15.16              | 18.82 | 14.51 | 23.07 | 18.51 $\pm$ 1.3 |

Table 110. Mean milk production, percentage milk fat and CMT score of shaded and unshaded groups taken at weekly intervals

| Day     | THI <sup>a</sup> | Milk (Kg) |        | Milk Fat (%) |        | CMT <sup>b</sup> |        |
|---------|------------------|-----------|--------|--------------|--------|------------------|--------|
|         |                  | Unshaded  | Shaded | Unshaded     | Shaded | Unshaded         | Shaded |
| Sept. 4 | 75.6             | 17.14     | 21.32  | 3.02         | 3.38   | 3.00             | 2.00   |
| 11      | 75.2             | 16.98     | 20.67  | 3.42         | 3.74   | 3.25             | 2.60   |
| 18      | 74.8             | 17.38     | 21.03  | 3.62         | 3.80   | 2.25             | 2.20   |
| 25      | 74.9             | 16.94     | 19.97  | 3.52         | 3.64   | 2.25             | 2.40   |
| Oct. 1  | 73.9             | 16.14     | 18.20  | 3.47         | 3.82   | 2.25             | 2.40   |
| 9       | 75.0             | 14.08     | 18.61  | 3.52         | 3.74   | 3.00             | 2.20   |
| 15      | 75.3             | 14.30     | 17.95  | 3.67         | 4.14   | 3.00             | 2.60   |
| 23      | 74.1             | 15.30     | 18.64  | 3.92         | 4.10   | 2.75             | 2.40   |
| 29      | 73.9             | 13.70     | 17.35  | 4.05         | 3.52   | 3.25             | 2.80   |
| Nov. 5  | 73.3             | 14.80     | 17.30  | 4.25         | 4.30   | 3.25             | 2.60   |
| 12      | 74.2             | 14.08     | 17.61  | 4.25         | 3.68   | 3.00             | 3.00   |
| 19      | 71.8             | 13.49     | 17.70  | 3.85         | 4.34   | 3.50             | 3.20   |
| 25      | 69.9             | 13.37     | 17.73  | 4.07         | 3.80   | 3.25             | 3.00   |
| Dec. 4  | 71.5             | 13.42     | 15.95  | 4.42         | 3.98   | 3.00             | 2.80   |
| 11      | 71.3             | 13.32     | -----  | 4.50         | -----  | -----            | -----  |
| 18      | 70.0             | 12.90     | -----  | 4.05         | -----  | -----            | -----  |
| 25      | 66.9             | 13.82     | -----  | 4.20         | -----  | -----            | -----  |
| 31      | 66.7             | 13.52     | -----  | -----        | -----  | -----            | -----  |

<sup>a</sup>Average of THI of the milk sampling day and the 2 days that preceded it.

<sup>b</sup>California mastitis test. Data were coded. Negative, trace, 1<sup>+</sup>, 2<sup>+</sup>, and 3<sup>+</sup> were 1.0, 2.0, 3.0, 4.0, and 5.0 respectively.

Table 111. Monthly conception rate at MX plotted with monthly maximum and minimum temperature and humidity for 1963, 1964 and 1965

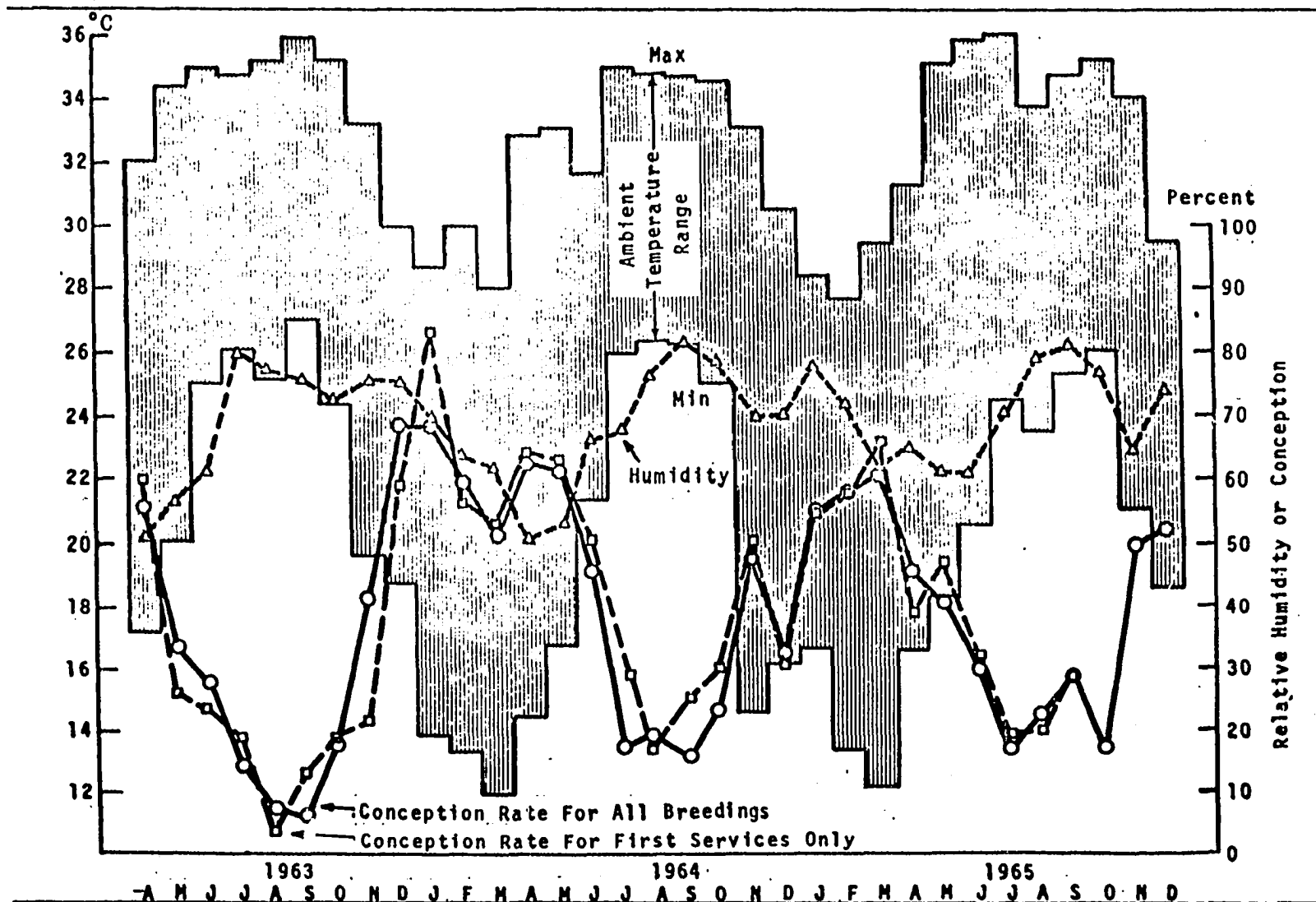


Table 112. Average THI vs. milk production for MX. The dashed line gives the actual daily milk production averaged for each month from daily total milk production records. Corrected milk production was computed from monthly milk production records on individual cows. It is the level of production that would have been obtained if the milking herd was composed of equal numbers of cows in each of the first 10 months of lactation

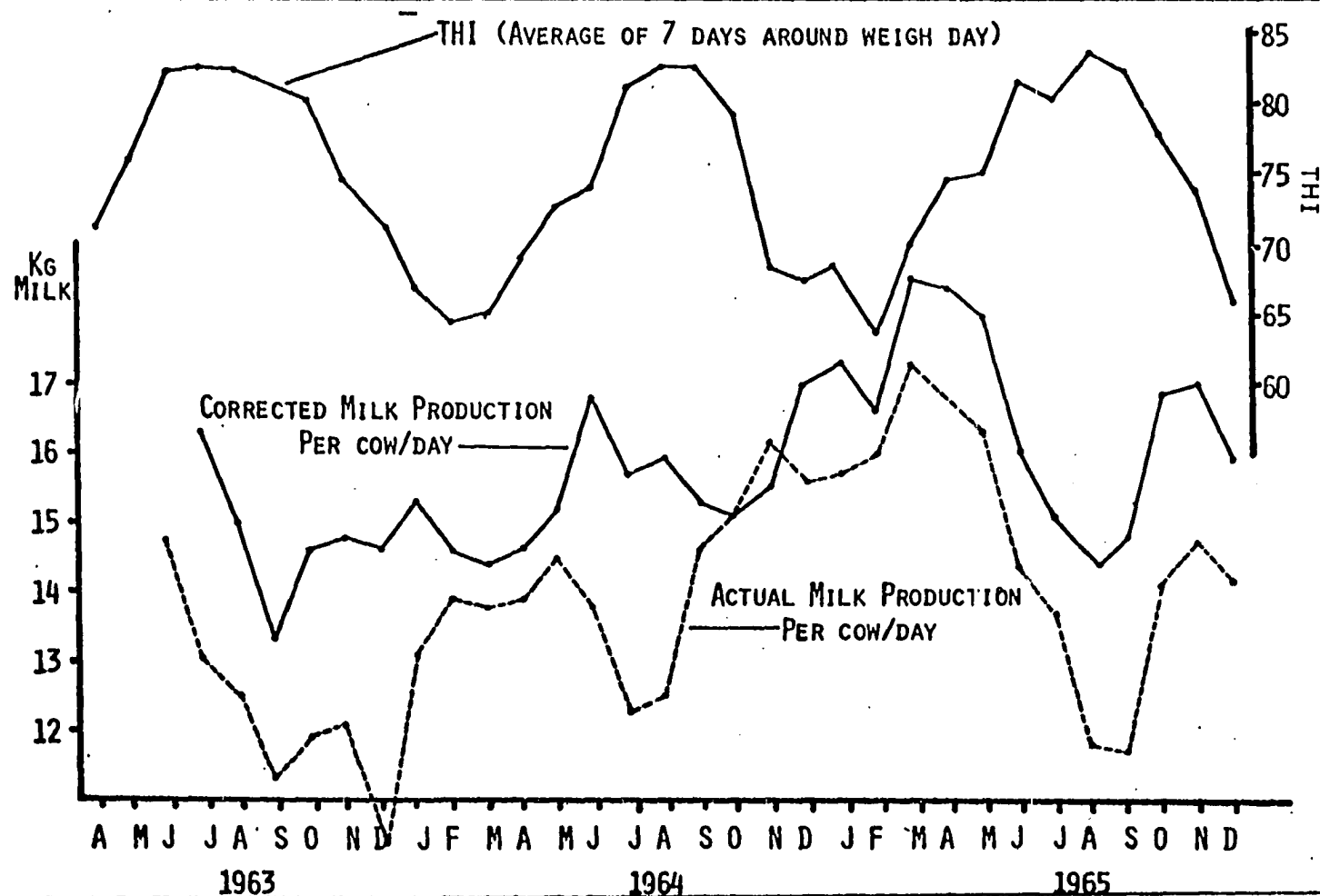


Table 113. Linear correlation coefficients<sup>a</sup>, for weather, conception rate and milk production<sup>b</sup>

|            | Conception | Min. Temp. | Max. Temp. | Avg. Temp. | Humidity | Avg. THI | Production |
|------------|------------|------------|------------|------------|----------|----------|------------|
| Conception | 1.000      | -0.738     | -0.565     | -0.711     | -0.521   | -0.756   | 0.635      |
| Min. Temp. | -0.738     | 1.000      | 0.718      | 0.943      | 0.578    | 0.947    | -0.639     |
| Max. Temp. | -0.565     | 0.718      | 1.000      | 0.838      | 0.037    | 0.766    | -0.276     |
| Avg. Temp. | -0.711     | 0.943      | 0.838      | 1.000      | 0.436    | 0.971    | -0.590     |
| Humidity   | -0.521     | 0.578      | 0.037      | 0.436      | 1.000    | 0.555    | -.0733     |
| Avg. THI   | -0.756     | 0.947      | 0.766      | 0.971      | 0.555    | 1.000    | -0.653     |
| Production | 0.635      | -0.639     | -0.276     | -0.590     | -0.733   | -0.653   | 1.000      |

<sup>a</sup>Coefficients greater than 0.357 ( $P < .001$ ).

<sup>b</sup>Based on data representing mean values for 87 consecutive 7-day periods between March, 1964 and November, 1965, from cattle in Mexico (MX). This is part of the period covered in Tables 111 and 112.

Table 114. The relationships between TDN consumed, milk production and average rectal temperature (0230 and 1330 rectal temperature averaged) in a group of 25 cows over a 20-day period of changing weather at MX. Data are from Ingraham (1968)

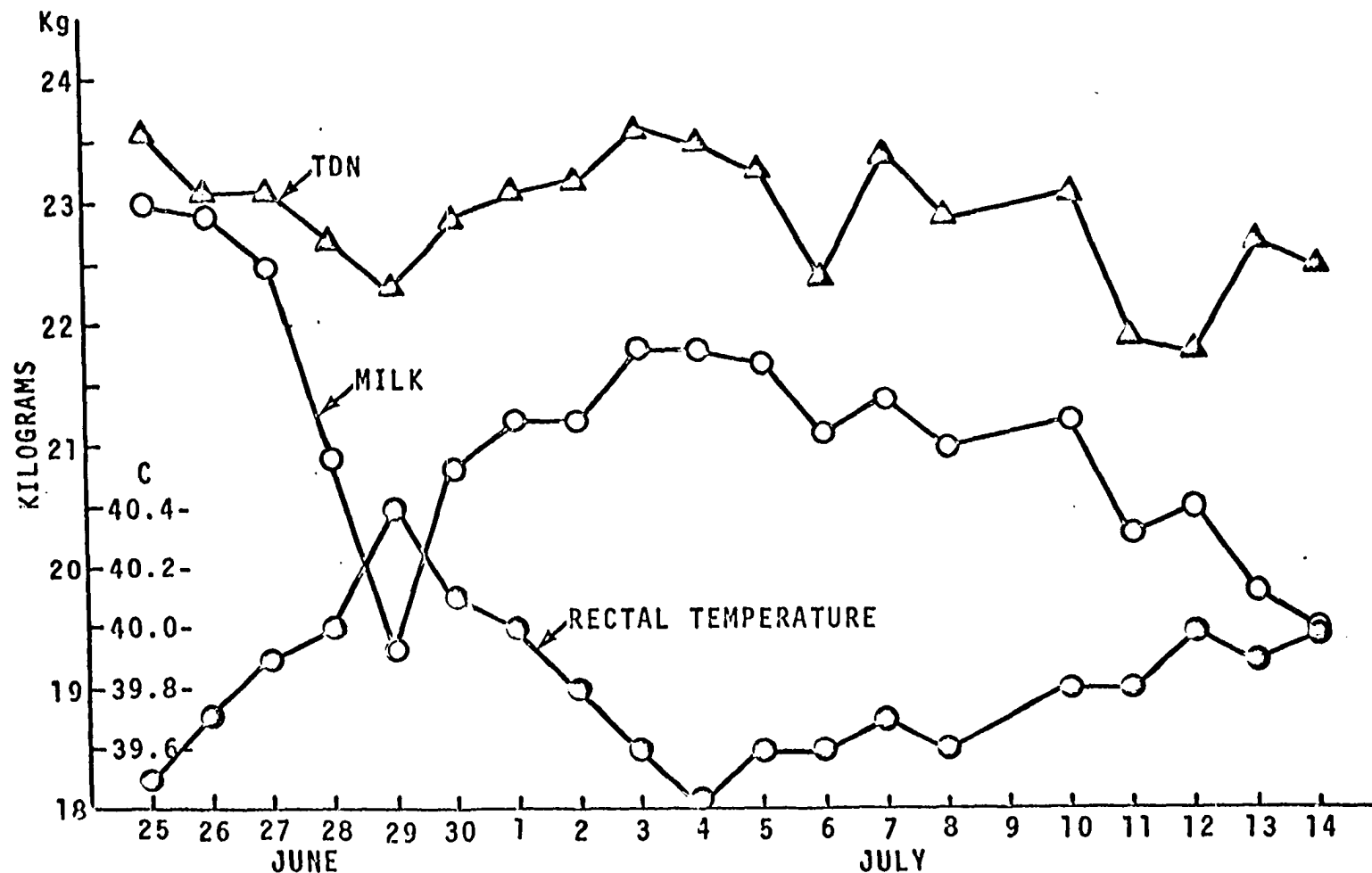


Table 115. Cumulative distribution curves for rectal temperature (average of 0230 and 1330) of 49 cows at average daily THI of 76.6, 78.5 and 80.3 (from Ingraham, 1968)

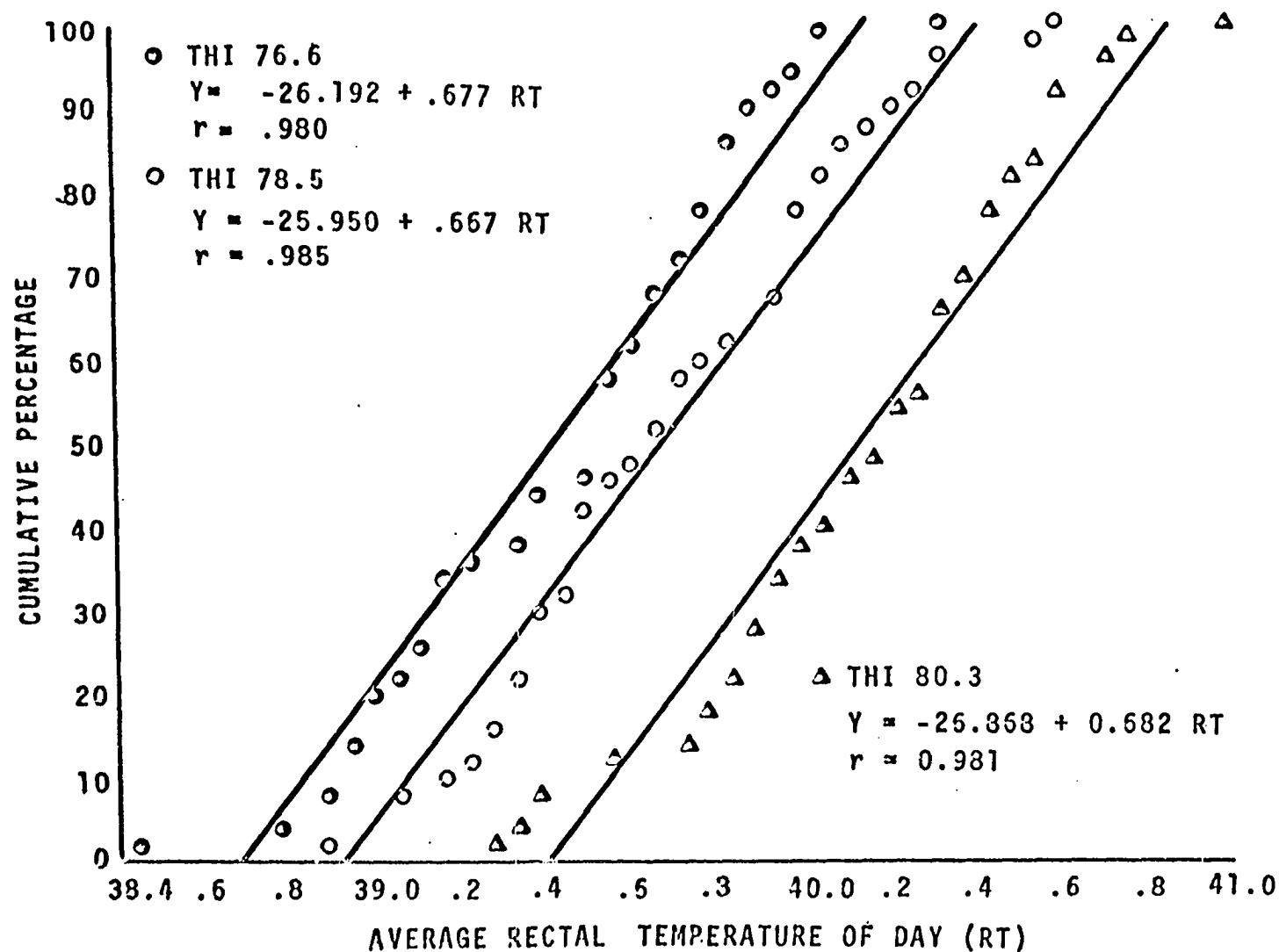




Table 116. Average number of days ( $\pm$ SD) from calving to first service for cattle at HW

|      | Jan.-Feb.                     | Mar.-April        | May-June          | July-Aug.         | Sept.-Oct.        | Nov.-Dec.         | Avg.               |
|------|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| 1965 | 116 $\pm$ 44 (3) <sup>a</sup> | 138 $\pm$ 72 (9)  | 87 $\pm$ 32 (4)   | 109 $\pm$ 67 (33) | 110 $\pm$ 49 (56) | 98 $\pm$ 38 (103) | 105 $\pm$ 49 (208) |
| 1966 | 85 $\pm$ 33 (102)             | 76 $\pm$ 22 (61)  | 90 $\pm$ 42 (23)  | 94 $\pm$ 61 (82)  | 87 $\pm$ 39 (98)  | 84 $\pm$ 40 (91)  | 86 $\pm$ 41 (457)  |
| 1967 | 79 $\pm$ 29 (141)             | 86 $\pm$ 33 (120) | 82 $\pm$ 34 (115) | 77 $\pm$ 33 (139) | 76 $\pm$ 39 (129) | 92 $\pm$ 46 (148) | 82 $\pm$ 37 (792)  |
| 1968 | 102 $\pm$ 44 (157)            | 87 $\pm$ 45 (165) | 82 $\pm$ 27 (150) | 75 $\pm$ 26 (182) | 61 $\pm$ 18 (79)  | 51 $\pm$ 5 (3)    | 83 $\pm$ 37 (736)  |
| Avg. | 90 $\pm$ 37 (403)             | 86 $\pm$ 40 (355) | 82 $\pm$ 33 (292) | 82 $\pm$ 42 (436) | 81 $\pm$ 40 (362) | 91 $\pm$ 43 (345) | 85 $\pm$ 41 (2193) |

<sup>a</sup>Number of intervals represented.

Table 117. Average number of days ( $\pm$ SD) between first and second service at HW

|      | Jan.-Feb.         | Mar.-April                   | May-June          | July-Aug.         | Sept.-Oct.        | Nov.-Dec.         | Avg.               |
|------|-------------------|------------------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| 1965 | -----             | 104 $\pm$ 0 (1) <sup>a</sup> | 128 $\pm$ 105 (4) | 102 $\pm$ 58 (4)  | 101 $\pm$ 58 (21) | 66 $\pm$ 34 (22)  | 88 $\pm$ 59 (52)   |
| 1966 | 56 $\pm$ 30 (30)  | 38 $\pm$ 28 (63)             | 39 $\pm$ 23 (35)  | 56 $\pm$ 44 (12)  | 48 $\pm$ 29 (29)  | 51 $\pm$ 36 (39)  | 46 $\pm$ 31 (208)  |
| 1967 | 43 $\pm$ 32 (53)  | 43 $\pm$ 31 (72)             | 37 $\pm$ 17 (59)  | 45 $\pm$ 32 (64)  | 40 $\pm$ 28 (77)  | 58 $\pm$ 46 (53)  | 44 $\pm$ 32 (378)  |
| 1968 | 48 $\pm$ 31 (54)  | 43 $\pm$ 26 (52)             | 46 $\pm$ 41 (100) | 52 $\pm$ 43 (90)  | 48 $\pm$ 40 (108) | 64 $\pm$ 37 (72)  | 50 $\pm$ 39 (471)  |
| Avg. | 48 $\pm$ 31 (138) | 41 $\pm$ 30 (188)            | 43 $\pm$ 39 (198) | 51 $\pm$ 41 (170) | 50 $\pm$ 41 (230) | 60 $\pm$ 39 (186) | 49 $\pm$ 38 (1109) |

<sup>a</sup>Number estrous cycles represented.

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